

Open Research Online

The Open University's repository of research publications and other research outputs

Immunogenetic analysis of HLA Class II in premalignant disease of the cervix and correlation with HPV status

Thesis

How to cite:

Odunsi, Adekunle Omatayo (1999). Immunogenetic analysis of HLA Class II in premalignant disease of the cervix and correlation with HPV status. PhD thesis The Open University.

For guidance on citations see [FAQs](#).

© 1999 The Author



<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Version: Version of Record

Link(s) to article on publisher's website:

<http://dx.doi.org/doi:10.21954/ou.ro.0000d51c>

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

oro.open.ac.uk

**IMMUNOGENETIC ANALYSIS OF HLA CLASS II IN PREMALIGNANT
DISEASE OF THE CERVIX AND CORRELATION WITH HPV STATUS**

A thesis presented for the degree of Doctor of Philosophy, Ph.D.

**Sponsoring Establishment: Institute of Molecular Medicine, John Radcliffe
Hospital, Oxford.**

Adekunle Omotayo Odunsi, BSc MBChB MRCOG

June, 1998

**AWARDING BODY:
THE OPEN UNIVERSITY**

AUTHOR'S NUMBER : P91276335

DATE OF AWARD : 28 JANUARY 1999

PAGE/PAGES
EXCLUDED
UNDER
INSTRUCTION
FROM
UNIVERSITY

ABSTRACT

IMMUNOGENETIC ANALYSIS OF HLA CLASS II IN PREMALIGNANT DISEASE OF THE CERVIX AND CORRELATION WITH HPV STATUS

The human papilloma virus (HPV) infection has a causal association with cervical intra-epithelial neoplasia (CIN) and cervical cancer. However, pre-malignant or malignant transformation is not always observed with HPV infection. HLA molecules are important in the regulation of the immune response to foreign antigens. The role of genetic variation at the HLA class II loci (DR and DQ) in CIN was investigated in 176 British Caucasian patients and 420 controls (normal cervical cytology and negative for HPV 16, 18, 31 and 33). HLA DQB1*03 typing was performed by a novel polymerase chain reaction-restriction fragment length polymorphism method (A-RFLP). The technique uses PCR to mutate the first base of codon 40 (DQ alleles) from T to G to create an artificial restriction site for an enzyme, *MluI*, which distinguishes DQB1*03 from other alleles and is confirmed by digestion of amplified DNA with *MluI*. Further HLA DR-DQ typing was performed by PCR DNA amplification and oligonucleotide probe typing. HPV types (16, 18, 31 & 33) were detected by using type-specific oligonucleotide primers and PCR. The alleles of the DQB1*03, DRB1*04 and DRB1*11 groups were strongly associated with susceptibility to CIN. Specifically the haplotypes DRB1*0401-DQB1*0301 and DRB1*1101-DQB1*0301 were significant and indicated susceptibility. The DQB1*03 locus was more contributory to this association than the DRB1 loci. A weak protective effect was shown for the haplotype DRB1*0101-DQB1*0501. Positive correlation was also observed for HPV-positive CIN, suggesting that specific HLA alleles may be important in determining the immune response to HPV antigens and the risk for CIN after HPV infection. Immunoaffinity purification of the susceptibility and protective HLA DQ molecules was performed and the naturally processed peptides were eluted and sequenced by Edman degradation. The data obtained was used for motif prediction of HPV 16 E6, E7, L1 and L2 sequences that may be capable of binding to these HLA molecules. Motif

prediction as well as the binding affinity of predicted peptide motifs for HLA DRB1*0401 and DRB1*0101 was accomplished using the published data on the naturally bound peptide sequences bound to these HLA molecules. The results revealed significant differences in both the number and binding affinity of the HPV 16 derived peptides to the protective and susceptibility HLA molecules. These results should help in the rational design of vaccines against HPV.

DEDICATION

This work is dedicated to my wife, Ayo; and to our daughters, Tosin, Tomi and Tolu.

ACKNOWLEDGMENTS

I am indebted to a number of people for their support, encouragement, and friendship during my stay in the U.K.

Particularly, I am most grateful to Dr. Trivadi S. Ganesan for accepting me into his laboratory and for his guidance, encouragement, intellectual stimulation as well as his patience, support and understanding during both the experimental work and the writing of this thesis. In addition, he provided financial support after my funding in the lab had expired, help with fellowship applications, the opportunity to write a review article, and the unpleasant task of reading this thesis in its many forms. I thank him also for his advice on many issues (both scientific and personal), and for listening and lifting many difficult moments.

I am also grateful to Professor John Bell for his guidance, unfailing support and encouragement.

I am very grateful to Wellbeing and the Royal College of Obstetricians and Gynaecologists for providing me with a research fellowship and the Imperial Cancer Research Fund for allowing me to work in their laboratories.

I would also like to thank my former teachers, particularly, Dr. Gordon Mackay and Dr. Ralph Robinson, and my friends Dr. Niyi Bankole, Dr. Rotimi Odutayo and Yetunde Odutayo for their support, encouragement and many happy memories.

CONTRIBUTIONS

I would like to thank the following individuals and organizations for their contributions:

Drs. Jack Cuzick, A. Hollingworth, A. Szarewski and Prof. A. Singer for providing the clinical samples.

Drs. George Terry and Linda Ho for performing HPV typing and allowing the use of the Biomek 1000 equipment in their laboratory for HLA typing.

Drs. Robert Edwards and Peter Sasieni for help with statistical analysis of a very large data set.

Dr Robert. Winchester for providing anti-HLA DQ antibody, IA3.

Dr. Miles Davenport for advice with immunoaffinity purification of HLA DQB1*0301 and DQB1*0501.

Dr. Kathy Stone for help and advice with Edman sequencing.

Dr. Jonathan Rothbard for providing advice on peptide motif prediction and for allowing the use of his DRB1*0101 and DRB1*0401 prediction database.

CONTENTS

| | | |
|-------------------------------|---|-------------|
| TITLE | PAGE | I |
| ABSTRACT | | II |
| DEDICATION | | I V |
| ACKNOWLEDGMENT | | V |
| CONTRIBUTIONS | | V I |
| CONTENTS | | V II |
| LIST OF TABLES | | XIII |
| LIST OF FIGURES | | X V |
| CHAPTER 1 INTRODUCTION | | |
| 1.1 | CERVICAL CANCER | 3 |
| 1.1.1 | GENERAL INTRODUCTION | 3 |
| 1.1.2 | PATHOGENESIS | 3 |
| 1.1.3 | ETIOLOGY | 6 |
| 1.2 | HUMAN PAPILLOMAVIRUS | 7 |
| 1.2.1 | GENERAL PROPERTIES | 7 |
| 1.2.2 | HPV GENOME ORGANIZATION | 9 |
| 1.2.3 | HPV DETECTION METHODS | 11 |
| 1.2.4 | PAPILLOMAVIRUSES AS CAUSATIVE AGENTS IN CERVICAL NEOPLASIA | 12 |
| 1.2.5 | MOLECULAR MECHANISMS OF HPV IN CERVICAL ONCOGENESIS | 14 |
| 1.2.5.1 | The E7 Gene | 14 |
| 1.2.5.2 | The E6 Gene | 16 |
| 1.2.5.3 | The E5 and E2 Genes | 18 |
| 1.2.5.4 | Physical State of Viral DNA | 19 |

| | | |
|---------|---|----|
| 1.2.6 | SUMMARY OF EVIDENCE FOR THE ROLE OF HPV IN CERVICAL ONCOGENESIS..... | 20 |
| 1.3 | THE HUMAN IMMUNE SYSTEM..... | 20 |
| 1.3.1 | ACTIVATION OF THE IMMUNE RESPONSE..... | 21 |
| 1.3.2 | HUMORAL IMMUNE RESPONSE..... | 22 |
| 1.3.3 | CELLULAR IMMUNE RESPONSE..... | 22 |
| 1.3.3.1 | T cell mediated cytotoxicity..... | 23 |
| 1.4 | THE HLA COMPLEX..... | 24 |
| 1.4.1 | CLASS I AND CLASS II HLA MOLECULES..... | 26 |
| 1.4.1.1 | Viral Antigen Processing and Presentation: Class I Pathway..... | 28 |
| 1.4.1.2 | Viral Antigen Processing and Presentation: Class II Pathway..... | 30 |
| 1.4.2 | HLA DNA TYPING STRATEGIES..... | 32 |
| 1.5 | IMMUNE RESPONSE TO HUMAN PAPILLOMAVIRUS..... | 37 |
| 1.6 | HLA ASSOCIATIONS WITH HUMAN PAPILLOMAVIRUS AND CERVICAL CANCER..... | 39 |
| 1.7 | SIGNIFICANCE OF HLA ASSOCIATIONS WITH DISEASE: REVERSE IMMUNOGENETICS..... | 40 |
| 1.8 | APPROACHES TO DEFINING HLA CLASS II BINDING MOTIFS..... | 42 |
| 1.8.1 | The Use of Large Peptide Repertoires to Identify General HLA Class II motifs..... | 42 |
| 1.8.2 | The Use of Single-Substitution Experiments on Naturally Processed Peptides to Identify Specific HLA Class II-Binding Motifs..... | 44 |
| 1.8.3 | The Use of Quantitative Matrices to Identify HLA Class II motifs..... | 45 |
| 1.9 | AIMS OF THE THESIS..... | 46 |

CHAPTER 2: METHODS AND MATERIALS

| | | |
|------------|---|-----------|
| 2.1 | SAMPLE COLLECTION AND GENOMIC DNA EXTRACTION..... | 59 |
| 2.1.1 | SAMPLE COLLECTION..... | 59 |
| 2.1.2 | GENOMIC DNA PREPARATION..... | 59 |
| 2.2 | HPV TYPING..... | 60 |
| 2.2.1 | POLYMERASE CHAIN REACTION WITH TYPE SPECIFIC PRIMERS..... | 60 |
| 2.2.1.1 | PCR AMPLIFICATION CONDITIONS..... | 60 |
| 2.2.1.2 | PCR TEMPERATURE CONDITIONS..... | 60 |
| 2.2.1.3 | AGAROSE GEL ELECTROPHORESIS..... | 61 |
| 2.3 | HLA DQB1*03 TYPING: ARTIFICIAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (ARFLP)..... | 61 |
| 2.3.1 | PRINCIPLES OF ARFLP..... | 61 |
| 2.3.2 | DESIGN OF PRIMERS FOR ARFLP..... | 62 |
| 2.3.3 | PCR AMPLIFICATION CONDITIONS..... | 63 |
| 2.3.4 | RESTRICTION ANALYSIS..... | 63 |
| 2.4 | POLYMERASE CHAIN REACTION WITH SEQUENCE SPECIFIC PRIMERS (PCR-SSP) FOR HLA DQB1*03 SUBTYPING..... | 64 |
| 2.4.1 | PRINCIPLES OF PCR-SSP..... | 64 |
| 2.4.2 | PRIMERS FOR AMPLIFICATION OF DQB1*03 ALLELES..... | 64 |
| 2.4.3 | PCR AMPLIFICATION CONDITIONS..... | 64 |
| 2.5 | POLYMERASE CHAIN REACTION-DIGOXIGENIN LABELLED OLIGONUCLEOTIDE HYBRIDIZATION FOR HLA DQ-DR TYPING..... | 65 |
| 2.5.1 | INTRODUCTION AND PRINCIPLES..... | 65 |
| 2.5.2 | DIGOXIGENIN LABELING OF SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBES..... | 66 |
| 2.5.3 | HLA DQB GENERIC AMPLIFICATION..... | 67 |

| | | |
|----------|--|----|
| 2.5.4 | HLA DRB GENERIC AMPLIFICATION..... | 68 |
| 2.5.5 | PREPARATION OF DOT BLOTS..... | 68 |
| 2.5.6 | PREHYBRIDIZATION / HYBRIDIZATION AND TMACI WASHES..... | 69 |
| 2.5.7 | CHEMILUMINESCENT DETECTION OF DIGOXIGENIN LABELLED PROBES WITH CSPD..... | 70 |
| 2.5.8 | STRIPPING OF MEMBRANES..... | 71 |
| 2.5.9 | HLA DRB GROUP SPECIFIC AMPLIFICATION..... | 71 |
| 2.5.10 | SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DQB TYPING..... | 73 |
| 2.5.10.1 | HLA DQB PROBE SPECIFICITY..... | 74 |
| 2.5.11 | SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DRB TYPING..... | 74 |
| 2.5.11.1 | HLA DR PROBE SPECIFICITY..... | 75 |
| 2.6 | HAPLOTYPIC ASSIGNMENT..... | 79 |
| 2.7 | STATISTICAL ANALYSIS..... | 79 |
| 2.8 | CELL LINES AND CULTURE CONDITIONS..... | 79 |
| 2.8.1 | JHF CELL LINE..... | 80 |
| 2.8.2 | JESTHOM CELL LINE..... | 80 |
| 2.8.3 | CELL CULTURE CONDITIONS..... | 80 |
| 2.9 | IMMUNOAFFINITY PURIFICATION..... | 80 |
| 2.9.1 | ANTIBODY FOR IMMUNOAFFINITY PURIFICATION..... | 81 |
| 2.9.2 | TECHNIQUE OF AFFINITY CHROMATOGRAPHY..... | 81 |
| 2.10 | PEPTIDE ELUTION..... | 82 |
| 2.11 | SEPARATION OF PEPTIDES: REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (r-HPLC)..... | 82 |
| 2.12 | PEPTIDE SEQUENCING..... | 84 |

| | | |
|--------|--------------------------------------|----|
| 2.12.1 | EDMAN DEGRADATION..... | 85 |
| 2.13 | SOLUTIONS AND REAGENTS..... | 85 |
| 2.14 | DNA CONTROL KITS..... | 87 |
| 2.15 | CHEMICAL AND MATERIAL SUPPLIERS..... | 87 |

CHAPTER 3: ASSOCIATION BETWEEN HLA DQB1*03 AND CERVICAL INTRA-EPITHELIAL NEOPLASIA

| | | |
|-----|--|-----|
| 3.1 | INTRODUCTION..... | 97 |
| 3.2 | RESULTS..... | 98 |
| 3.3 | OVERALL RESULTS (APPENDIX 1)..... | 102 |
| 3.4 | ASSOCIATION BETWEEN HLA DQB1*03 AND CIN..... | 102 |
| 3.5 | ASSOCIATION BETWEEN HLA DQB1*03 AND HPV..... | 103 |
| 3.6 | SUMMARY AND DISCUSSION..... | 104 |

CHAPTER 4: ANALYSIS OF HLA DR-DQ ASSOCIATIONS WITH HPV AND CERVICAL INTRA-EPITHELIAL NEOPLASIA

| | | |
|-----|---|-----|
| 4.1 | INTRODUCTION..... | 114 |
| 4.2 | RESULTS..... | 114 |
| 4.3 | CORRELATION BETWEEN INDIVIDUAL ALLELES OF HLA DRB1, DRB3, DRB4 AND DRB5 WITH CIN..... | 119 |
| 4.4 | CORRELATION BETWEEN INDIVIDUAL HLA DQB1 ALLELES AND CIN..... | 119 |
| 4.5 | CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL HLA DQB1 AND HLA DRB ALLELES AND HPV..... | 120 |
| 4.6 | CORRELATION BETWEEN HLA DR/DQ HAPLOTYPES AND CIN..... | 120 |
| 4.7 | CORRELATION BETWEEN SIGNIFICANT HLA DR/DQ HAPLOTYPES AND HPV..... | 121 |
| 4.8 | CORRELATION BETWEEN SIGNIFICANT HLA DR/DQ HAPLOTYPES AND HPV TYPE..... | 121 |

| | | |
|-----|-----------------------------|-----|
| 4.9 | SUMMARY AND DISCUSSION..... | 122 |
|-----|-----------------------------|-----|

CHAPTER 5: POOL SEQUENCING OF NATURALLY PROCESSED PEPTIDES BOUND TO HLA-DQB1*0301 AND DQB1*0501; PREDICTION OF PEPTIDE MOTIFS FROM HUMAN PAPILLOMAVIRUS TYPE 16

| | | |
|-----|---|-----|
| 5.1 | INTRODUCTION..... | 132 |
| 5.2 | ELUTED POOL SEQUENCE DATA FROM PEPTIDES ELUTED FROM HLA DQB1*0501 AND DQB1*0301..... | 133 |
| 5.3 | PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM L1, L2, E6 AND E7 OF HPV 16 TO HLA DQB1*0301 AND DQB1*0501..... | 141 |
| 5.4 | PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM L1, L2, E6 AND E7 OF HPV 16 TO HLA DRB1*0101 AND 0401..... | 149 |
| 5.5 | SUMMARY AND DISCUSSION..... | 157 |

CHAPTER 6: DISCUSSION

| | | |
|-------|---|-----|
| 6.1 | SUMMARY..... | 162 |
| 6.2 | DISCUSSION..... | 163 |
| 6.3 | MECHANISMS OF HLA CLASS II ASSOCIATION WITH HPV AND CIN..... | 170 |
| 6.4 | POLYMORPHIC STRUCTURAL FEATURES OF HLA-DQ MOLECULES ASSOCIATED WITH SUSCEPTIBILITY OR RESISTANCE TO HPV ASSOCIATED CIN..... | 173 |
| 6.5 | CLINICAL IMPLICATIONS : HLA TYPING AND VACCINE DEVELOPMENT..... | 179 |
| 6.6 | CONCLUSIONS..... | 184 |
| 6.7 | FUTURE STUDIES..... | 185 |
| 6.7.1 | HLA ASSOCIATION STUDIES..... | 185 |

| | |
|---|-----|
| 6.7.2 ASSOCIATION WITH HLA RELATED GENES AND P53..... | 185 |
| 6.7.3 IMMUNODOMINANCE AND ANTIGEN PRESENTATION..... | 186 |
| 6.7.4 VACCINATION TRIALS..... | 186 |

| | |
|-----------------|-----|
| REFERENCES..... | 188 |
|-----------------|-----|

APPENDICES

APPENDIX I.....FULL HLA DR-DQ RESULTS ON PATIENTS WITH CIN

APPENDIX II.....FULL HLA DR-DQ RESULTS ON CONTROL POPULATION

APPENDIX III.....FULL MOTIF PREDICTION FROM HPV 16 E6, E7, L1 AND

L2 FOR BINDING TO HLA DRB1*0101 AND DRB1*0401

PUBLICATIONS

LIST OF TABLES

| | |
|--|-----|
| 1.1 THE BETHESDA SYSTEM OF CLASSIFICATION OF SQUAMOUS ABNORMALITIES COMPARED WITH OTHER NOMENCLATURE..... | 47 |
| 1.2 HPV GENOTYPES FROM CUTANEOUS AND MUCOSAL LESIONS..... | 48 |
| 1.3 FUNCTION OF HPV GENE PRODUCTS..... | 49 |
| 1.4 HLA ASSOCIATIONS WITH CIN AND CERVICAL CANCER..... | 50 |
| 2.1 TYPE SPECIFIC PRIMER PAIRS USED FOR HPV AMPLIFICATION..... | 89 |
| 2.2 SEQUENCE SPECIFIC PRIMER PAIRS FOR TYPING THE HLADQB1*03 LOCUS..... | 90 |
| 3.1 SUMMARY OF DISTRIBUTION OF HLA DQB1*03..... | 107 |
| 3.2 ASSOCIATION BETWEEN HLA DQB1*03 AND CIN..... | 108 |
| 3.3 ASSOCIATION BETWEEN HLA DQB1*03 ALLELE AND CIN..... | 109 |
| 3.4 ASSOCIATION BETWEEN HLA DQB1*03 AND HPV TYPE..... | 110 |
| 3.5 ASSOCIATION BETWEEN HLA DQB1*03 ALLELE AND HPV..... | 111 |
| 3.6 ASSOCIATION BETWEEN HLA DQB1*03 ALLELE AND HPV..... | 112 |

| | | |
|-----|--|-----|
| 4.1 | CORRELATION BETWEEN INDIVIDUAL DRB1, DRB3, DRB4, DRB5 ALLELES AND CIN..... | 124 |
| 4.2 | CORRELATION BETWEEN INDIVIDUAL DQ ALLELES AND CIN..... | 126 |
| 4.3 | CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL DQB1 AND DRB1 ALLELES AND HPV..... | 127 |
| 4.4 | CORRELATION BETWEEN DR/DQ HAPLOTYPES AND CIN..... | 128 |
| 4.5 | CORRELATION BETWEEN SIGNIFICANT DR/DQ HAPLOTYPES AND HPV..... | 129 |
| 4.6 | CORRELATION BETWEEN SIGNIFICANT DRB1/DQB1 HAPLOTYPES AND HPV TYPE..... | 130 |
| 5.1 | SEQUENCING OF DQB1*0501 LIGANDS..... | 137 |
| 5.2 | SEQUENCING OF DQB1*0301 LIGANDS..... | 138 |
| 5.3 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-E7 TO HLADQB1*0301..... | 143 |
| 5.4 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-E7 TO HLADQB1*0501..... | 143 |
| 5.5 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-E6 TO HLADQB1*0301..... | 144 |
| 5.6 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-E6 TO HLADQB1*0501..... | 144 |
| 5.7 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-L1 TO HLADQB1*0301..... | 145 |
| 5.8 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-L1 TO HLADQB1*0501..... | 146 |
| 5.9 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-L2 TO HLADQB1*0301..... | 147 |

| | | |
|-------------|--|------------|
| 5.10 | THE PREDICTED PEPTIDE MOTIFS FROM HPV 16-L2 TO | |
| | HLADQB1*0301..... | 148 |
| 5.11 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-L1 TO HLADRB1*0101..... | 152 |
| 5.12 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-L1 TO DRB1*0401..... | 153 |
| 5.13 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-L2 TO DRB1*0101..... | 153 |
| 5.14 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-L2 TO DRB1*0401..... | 154 |
| 5.15 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-E7 TO DRB1*0101..... | 154 |
| 5.16 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-E7 TO DRB1*0401..... | 155 |
| 5.17 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-E6 TO DRB1*0101..... | 155 |
| 5.18 | THE PREDICTED BINDING AFFINITY OF PEPTIDES DERIVED FROM HPV | |
| | 16-E6 TO DRB1*0401..... | 156 |
| 6.1 | SUMMARY OF THE STRUCTURAL FEATURES OF HLA DQB1*03 AND | |
| | HLA DQB1*0501..... | 178 |

LIST OF FIGURES

| | | |
|------------|---|-----------|
| 1.1 | SCHEMATIC PRESENTATION OF THE HPV 16 GENOME..... | 52 |
| 1.2 | SCHEMATIC PRESENTATION OF THE MECHANISMS OF CELLULAR | |
| | AND HUMORAL IMMUNE RESPONSES..... | 53 |
| 1.3 | THE HLA GENE COMPLEX..... | 54 |
| 1.4 | STRUCTURE OF THE HLA MOLECULE..... | 55 |

| | | |
|-----|---|-----|
| 2.1 | PRINCIPLES OF A-RFLP..... | 91 |
| 2.2 | THE DESIGN OF A-RFLP PRIMERS..... | 92 |
| 2.3 | A-RFLP FOR HLA DQB1*03..... | 93 |
| 2.4 | SCHEMATIC PRESENTATION OF DIGOXIGENIN..... | 94 |
| 2.5 | PRINCIPLES OF EDMAN DEGRADATION..... | 95 |
| 3.1 | 4% METAPHOR AGAROSE GEL ELECTROPHORESIS SHOWING AMPLIFIED DNA WITH AND WITHOUT DIGESTION BY <i>MluI</i> | 98 |
| 3.2 | EXAMPLE OF A-RFLP ON STUDY SAMPLES..... | 99 |
| 3.3 | PCR PRODUCTS OBTAINED BY HLA DQB1*03 SEQUENCE SPECIFIC PRIMERS..... | 100 |
| 3.4 | TYPE SPECIFIC AMPLIFICATION OF HPV..... | 101 |
| 4.1 | GENERIC HLA DQB1 AMPLIFICATION..... | 115 |
| 4.2 | GENERIC HLA DRB1 AMPLIFICATION..... | 116 |
| 4.3 | DOT BLOT OF HLA DQB1..... | 117 |
| 4.4 | DOT BLOT OF HLA DRB1..... | 118 |
| 5.1 | 12% SDS-PAGE ANALYSIS AFTER IMMUNOAFFINITY PURIFICATION: HLA DQA1*0301-DQB1*0301 OBTAINED FROM THE JESTHOM CELL LINE..... | 134 |
| 5.2 | HLA-DQB1*0501 PEPTIDE POOLS SEPARATED BY r-HPLC..... | 135 |
| 5.3 | HLA-DQB1*0301 PEPTIDE POOLS SEPARATED BY r-HPLC..... | 136 |

CHAPTER 1: INTRODUCTION

1.1 CERVICAL CANCER

1.1.1 GENERAL INTRODUCTION

1.1.2 PATHOGENESIS

1.1.3 ETIOLOGY

1.2 HUMAN PAPILLOMAVIRUS

1.2.1 GENERAL PROPERTIES

1.2.2 HPV GENOME ORGANIZATION

1.2.3 HPV DETECTION METHODS

1.2.4 PAPILLOMAVIRUSES AS CAUSATIVE AGENTS IN CERVICAL NEOPLASIA

1.2.5 MOLECULAR MECHANISMS OF HPV IN CERVICAL ONCOGENESIS

1.2.5.1 The E7 Gene

1.2.5.2 The E6 Gene

1.2.5.3 The E5 and E2 Genes

1.2.5.4 Physical State of Viral DNA

1.2.6 Summary of evidence for the role of HPV in cervical oncogenesis

1.3 THE HUMAN IMMUNE SYSTEM

1.3.1 ACTIVATION OF THE IMMUNE RESPONSE

1.3.2 HUMORAL IMMUNE RESPONSE

1.3.3 CELLULAR IMMUNE RESPONSE

1.3.3.1 T cell mediated cytotoxicity

1.4 THE HLA COMPLEX

1.4.1 HLA DNA TYPING STRATEGIES

1.4.2 CLASS I AND CLASS II HLA MOLECULES

1.4.2.1 Viral Antigen Processing and Presentation: Class I Pathway

1.4.2.2 Viral Antigen Processing and Presentation: Class II Pathway

1.5 IMMUNE RESPONSE TO HUMAN PAPILLOMAVIRUS

1.6 HLA ASSOCIATIONS WITH HUMAN PAPILLOMAVIRUS AND CERVICAL CANCER

1.7 SIGNIFICANCE OF HLA ASSOCIATIONS WITH DISEASE

1.8 APPROACHES TO DEFINING HLA CLASS II BINDING MOTIFS

1.8.1 The Use of Large Peptide Repertoires to Identify General HLA Class II motifs

1.8.2 The Use of Single-Substitution Experiments on Naturally Processed Peptides to Identify Specific HLA Class II-Binding Motifs

1.8.3 The Use of Quantitative Matrices to Identify HLA Class II motifs

1.9 AIMS OF THE THESIS

1.1 CERVICAL CANCER

1.1.1 GENERAL INTRODUCTION

Cervical cancer constitutes a major health problem worldwide. Each year, there are approximately 465,000 new cases and in excess of 200,000 deaths from the disease¹. The areas with the maximum incidence are in Southern and Eastern Africa and Central and tropical South America. The risk in Western European and North American countries is considered relatively low at less than 10 new cases per 100,000 women annually. The rates are 10 to 20 times higher in some parts of Northeastern Brazil where lifetime cumulative risks can approach 10%². In the United Kingdom, there are approximately 3,000 new cases each year and over 2,000 deaths from the disease. Likewise, in the United States, each year there are approximately 16,000 new cases of invasive cervical cancer with 4800 deaths due to the disease³. For each new case of cervical cancer found by cytology screening, there are approximately 50 other cases of abnormal cervical smears that merit careful monitoring by colposcopic follow-up and eventually, biopsy. To this triage burden must be added an equal number of cases of borderline atypias (the so-called "ASCUS" smears) that are found concurrently and need to be confirmed by repeat cytology (MMWR, 1994). Although much effort has been applied to the early detection of cervical cancer by exfoliative cytology and the provision of conservative modalities of treatment for pre-invasive lesions, study of the molecular mechanisms of cervical carcinogenesis has only recently become an intense field of research.

1.1.2 PATHOGENESIS

The uterine cervix consists of the ectocervix, which is covered by glycogen rich squamous epithelium, and endocervix, lined with a single layer of columnar epithelial cells. Colposcopic and histologic examination of the cervix from perinatal to adult life discloses three epochs when columnar epithelium on the endocervix is activated by contact with the vaginal environment. These epochs, which correspond with periods of maximal estrogen

stimulation are perinatal, at the menarche, and during the first pregnancy⁴. As a result of contact with the lower pH of the vaginal environment, the columnar epithelium is induced to transform into stratified squamous epithelium. The new squamous epithelium soon becomes an undifferentiated 8 to 10 cell-thick structure composed of rounded cells. Later differentiation occurs into basal, intermediate and superficial cells. Finally, the mature epithelium comes to mimic closely the squamous epithelium of the vaginal portion of the cervix and of the vagina which it adjoins abruptly at a line running concentrically around the os. Within this line, the squamous epithelium is new and distinct from the original or native squamous epithelium outside its limits. Thus, the limits of the *transformation zone* lies between the original squamo-columnar junction (now squamo-squamous) and the new squamo-columnar junction. This site is thought to be where premalignant lesions of the cervix develop.

Cytologic examination of cervical smears is used to detect cervical abnormalities. Women with abnormal cervical (Papanicolaou) smears are subsequently examined by colposcopy. The lesions in the transformation zone that appear atypical by colposcopy are diagnosed by histological examination. Cervical lesions with the potential to progress to invasive cancer were originally histologically diagnosed as dysplasia. Cervical dysplasia is characterized by a disturbed epithelial architecture and cellular atypia. Depending on the proportion of the epithelial layer that shows dysplastic changes, lesions are classified as mild, moderate or severe dysplasia. In 1968, the concept of cervical intraepithelial neoplasia (CIN) was introduced⁵. In this scheme, CIN is a continuous spectrum of intraepithelial changes, that starts with minor atypia, progressing through increasing degrees of intra-epithelial abnormalities to invasive squamous cell carcinoma. CIN lesions are classified according to the thickness of the epithelial layer involved in neoplastic change. Involvement of the lower one third of the epithelial layer represents CIN grade 1, one third to two thirds involvement

CIN grade II, and two thirds to full thickness CIN grade III, which is equivalent to carcinoma in situ (CIS)⁶.

This classification scheme led to two major problems. Firstly, the relative high inter- and intra-observer variation in interpretation by pathologists⁷ and the attitude of the clinician toward treatment of CIN II lesions, with a choice between treatment and watchful expectancy. By 1988, several classifications and many modifications were in use throughout the world resulting in confusion in communications among clinicians, pathologists and researchers. The result of a workshop by the National Cancer Institute was the Bethesda System⁸ to replace the various Papanicolaou designations and to standardize cytologic terminology to correlate with histologic reports. A comparison between the Bethesda System and earlier ones is summarized in Table 1.1. The term CIN was replaced by Squamous Intraepithelial Neoplasia (SIL), with CIN II and III replaced by high grade SIL (HGSIL) while CIN I was replaced by low grade SIL (LGSIL). This suggests that CIN grade I lesions constitute a different entity from CIN II or III lesions, lacking the ability to progress to cervical cancer. In reality, about 20 - 30% of cervical lesions with mild dysplasia will progress to carcinoma in situ⁹⁻¹⁰. In another study, 50% of patients with CIN I progressed to CIN III, while 25% either progressed to CIN II or remained at grade I for nine years¹¹. In the United Kingdom, approximately 250,000 women are diagnosed annually with CIN. The much lower frequency of cervical cancer per year suggests that only a proportion of lesions diagnosed as CIN will progress to carcinoma.

The mean age for cervical cancer is 52.2 years, and the distribution is bimodal, with peaks at 35-39 years and 60-64 years¹². The incidence of adenocarcinoma appears to be increasing relative to that of squamous cancers. Older reports indicate that 5% of all cervical cancers were adenocarcinomas¹³, whereas newer reports suggest an incidence as high as

18.5-27%¹⁴⁻¹⁵. There is evidence to suggest a poorer prognosis for adenocarcinoma than for squamous cell carcinoma in every stage. Hopkins & Morley¹⁵ performed a Cox proportional hazard analysis of 203 women with adenocarcinoma and 756 women with squamous carcinoma and reported 5-year survival rates of 90% versus 60%, 62% versus 47%, and 36% versus 8% for stages I, II, and III, respectively. Although this has been attributed to a relative resistance to radiation, it is more likely a reflection of the tendency of adenocarcinomas to grow endophytically and to be undetected until a larger volume of tumour is present. It is also unclear whether or not patients with adenosquamous carcinoma of the cervix have a poorer prognosis than those with pure adenocarcinoma or squamous carcinoma¹⁶⁻¹⁷.

1.1.3 ETIOLOGY

The epidemiological pattern of cervical cancer strongly points to a sexually transmitted infectious agent as being etiologically important. As early as 1842, Rigoni Stern¹⁸ found that compared with breast cancer, cervical cancer was eighteen times more common amongst married than unmarried women in Verona. Modern epidemiological studies have shown low cervical cancer rates among catholic nuns¹⁹ while higher rates are found in women marrying at younger ages²⁰⁻²¹. The latter is related more specifically with two aspects of sexual behaviour namely number of sexual partners and age at initiation of intercourse²²⁻²³. The risk associated with ten or more partners is nearly three times higher than that associated with one or fewer partners²⁴⁻²⁵. Women with first sexual experiences before age 16 have about twice the risk compared with women who initiate sexual intercourse after the age of 20 years and this may reflect the increased susceptibility of the "younger" cervix to carcinogenic insult²⁶. Other risk factors include cigarette smoking and the use of the oral contraceptive pill.

Different candidates for a sexually transmitted agent have been proposed including syphilis, gonorrhea, *Trichomonas vaginalis* and Herpes simplex virus type 2 (HSV-2). In particular, HSV-2 appeared a plausible candidate, because of the high frequency of HSV-2 specific antibodies in cervical cancer patients compared with healthy controls. Although HSV was proven to be carcinogenic, in vitro and in vivo clinical studies eventually demonstrated that only a fraction of cervical carcinomas contained traces of HSV viral DNA, and epidemiological studies failed to demonstrate an association between HSV and cervical cancer²⁷⁻²⁸.

In 1976, zur Hausen hypothesized that cervical cancer shows a similar epidemiological pattern as genital warts²⁹. The formation of genital warts (condylomata acuminata) was considered to be associated with infection with the human papillomavirus (HPV). Subsequently, zur Hausen and Gissman using recombinant DNA technology were able to clone and characterize isolated HPV DNA from genital warts and papillomas³⁰⁻³². Novel HPV types in cervical cancer were identified that differed from those associated with genital warts. Since then, there have been several compelling epidemiological, clinical, and molecular biologic data indicating that the infectious agent with a causal relationship to CIN and cancer is the human papillomavirus (discussed below).

1.2 HUMAN PAPILLOMAVIRUS (HPV)

1.2.1 GENERAL PROPERTIES

Papillomavirus belong to the family Papovaviridae. They possess a closed, circular, double stranded genome and are encapsulated in an icosahedral capsid of about 55nm in diameter, consisting only of protein. Their genome is approximately 7.9kB in size and more than 70 different types of papillomaviruses have been identified, many of which infect the anogenital epithelium. By definition, the different types share less than 50% homology under stringent conditions³³⁻³⁴. Since the number of complete sequences of different HPV types

is rapidly increasing, and many more HPVs are being identified, a modified definition of a new HPV type has been proposed³⁵. Here, a new type is defined when less than 90% sequence homology in E6, E7 and L1 region is found with any other known HPV type.

Two major groups are defined according to their epithelial affinity - types infecting the dry skin and those infecting the moist mucosal areas of the body (Table 1.2). The genital HPV types are placed into three broad categories based on the frequency of association with malignant tumors and thus the presumed oncogenic potential. The low risk groups includes types 6, 11, 42 and 44, which are common in LGSIL and less so in HGSIL and practically non existent in cancer specimens. The intermediate risk group is comprised of types 31, 33, 35, 51 and 52 whose combined frequency of association increase within the SIL spectrum but decrease in carcinomas. The high risk group includes HPV 16, 18, 45 and 56, which are strongly associated with carcinomas and exhibit diverse behaviour with respect to HGSIL³⁶.

HPVs infect the basal cells of the epithelium and rather than proceeding to a lytic infection in which viral replication kills the cell, viral DNA transcription and replication are maintained at very low levels (20-100 copies per cell) until more superficial epithelium is infected. At this level, viral transcription accelerates, DNA synthesis begins and virions assemble³⁷. In addition, the activation of late gene expression and capsid assembly occurs concurrently with the amplification of viral DNA. In benign or pre-malignant lesions, the HPV DNA exists extra-chromosomally as a plasmid. By contrast, all cervical cancer derived cell lines that contain HPV DNA and the majority of primary tumours reveal integrated viral DNA³⁸.

1.2.2 HPV GENOME ORGANIZATION

The HPV genome consists of 3 regions : one regulatory non coding region, termed the long control region (LCR), and two coding regions (Fig 1.1). The ' early ' region consists of six to eight open reading frames , whereas the ' late ' region encodes two genes. By definition, early genes are expressed shortly after viral infection and prior to viral replication. The late genes code for the structural proteins forming the viral particle, and are expressed in the late phase of infection. The functions of the different reading frames are summarized in table 1.3.

E1: HPV E1 is homologous with polyoma large T domains³⁹ and BPV-E1⁴⁰. The viral ring molecule is usually opened within the 3' end of the E1 or the 5' end of the E2 open reading frames and mutations in the E1 or E2 gene increases HPV 16 immortalisation efficiency in keratinocytes⁴¹. In monolayer cultures, both E1 and E2 gene products are required for the transient replication of viral genomes⁴²⁻⁴³ and the origin of replication (ori) maps to a region adjacent to the E6 open reading frame (ORF), which binds the E1 protein. The E1 ORF also encodes a nuclear phosphoprotein that can bind the E2 molecule⁴⁴. While the E1 protein may bind DNA by itself⁴⁵, the presence of E2 increases its affinity for binding⁴⁶.

E2: The E2 protein acts as a transcriptional trans-activator, via the E2 responsive elements (E2-RE), located in the LCR⁴⁷. E2 disruption is thought to alter regulation of expression of E6 and E7 genes⁴⁸. In high risk HPVs, the primary transcriptional activity of E2 appears to be as a repressor E6 and E7 transcription⁴⁹⁻⁵⁰. Two E2 sites are located 2 to 4 bp upstream from the putative TATA box and this close proximity may result in steric hinderance by bound E2 proteins.

E4: The E4 gene product is expressed as an E1-E4 fusion protein in the "late" phase of the viral life cycle. In this form, it disrupts keratin assembly in stratified suprabasal cells, allowing for viral egress⁵¹⁻⁵².

E5: The HPV E5 encodes a membrane protein with weak transforming activity⁵³. Part of its transforming function may reside in its ability to complex with epidermal growth factor (EGF) receptor, resulting in an enhanced EGF-mediated signal transduction to the nucleus, as shown by increased *c-fos* expression levels⁵⁴.

E6 and E7: Analysis of the transcription of HPV 16 in cervical cancer derived cell lines and in fresh premalignant and malignant cervical biopsies have shown that transcription of the E6 - E7 region of the HPV 16 genome is selectively retained in the neoplastic tissue⁵⁵⁻⁵⁶. E6 and E7 have been identified as the transforming genes of HPV16 and it appears that both are necessary for full transformation of cervical epithelial cells⁵⁷⁻⁵⁸.

The E6 and E7 gene products of HPV 16 & 18 can each transform immortalized rodent cells such as NIH 3T3 and Rat-1 cells to an anchorage dependent state⁵⁹. The E7 proteins can also transform primary rodent cells, but require the additional presence of an activated ras gene for full activity⁶⁰. In the human keratinocyte, the HPV E6 and E7 act in a co-operative fashion to efficiently immortalize cells⁶¹⁻⁶². The E7 gene by itself can immortalize keratinocytes at low frequency, whereas the E6 protein does not exhibit any such properties. Furthermore, keratinocytes immortalized by HPV 16 and 18 E6/E7 only become tumourigenic by the addition of activated ras gene or following prolonged passage in culture⁶³.

L1 and L2: The L1 and L2 open reading frames encode the major and minor capsid proteins respectively.

LCR: The LCR is located between the early and late genes (also termed upstream regulatory region, URR), and contains viral gene promoter and enhancer sequences which are dependent solely on cellular factors for function⁶⁴. These enhancers are called constitutive or "C" enhancers for HPV 11, 16 and 18 and are located in the URR 200 to 300bp upstream of the E6 ORF. C enhancers direct expression of heterologous promoters preferentially in cell lines derived from squamous cell carcinomas, as well as in primary human keratinocytes. Transcripts controlled by these enhancers initiate upstream of the E6 gene at a promoter referred to by nucleotide number p97 in HPV 16 and HPV 31 and p101 in HPV 31. These enhancers include AP-1, keratinocyte specific factor, KRF-1⁶⁵ and steroid receptors^{11,66}.

1.2.3 HPV DETECTION METHODS

Classical viral cultivation techniques are not applicable in HPV because the virus cannot be propagated in culture. Consequently, in order to assess the presence of HPV in clinical samples, HPV DNA detection methods using hybridization based techniques have been developed. Southern blot analysis allows highly specific HPV genotype detection with a sensitivity range between 0.1 and 0.01 HPV genome copies per cell. However, the methodology is too time-consuming and labour intensive making it unsuitable for mass screening purposes. Other methods like dot blot analysis, *in situ* hybridization and filter *in situ* hybridization, are less labour intensive, but suffer from other drawbacks such as reduced specificity and sensitivity.

The advent of the polymerase chain reaction (PCR) has considerably increased the possibility of screening a large number of samples⁶⁷⁻⁶⁸. The method is superior in sensitivity as compared to other HPV detection techniques, with a sensitivity of 1 copy per 10⁶ cells in a clinical sample being detectable⁶⁹, and it requires low amounts of target

DNA. Furthermore, it can be applied directly to crude cell extracts without the need of DNA isolation from every clinical sample⁷⁰ and can also be applied on fixed tissue⁷¹⁻⁷².

Many alternative sets of primers are used for detecting HPV by PCR. Two commonly used sets are the consensus primer pair MY09 and MY11⁷³ and the general primers GP5 and GP6⁶⁹. In both systems, the primers used are homologous with sequences in the L1 ORF of HPVs, since this region is highly conserved. Several other sets of primers have been reported in the literature, such as consensus E1 primers, but their use is not widespread enough. In the MY 09/11 system, a PCR amplicon of about 450 nucleotides is produced and this can be subsequently typed by dot blotting of PCR products using radioactive or biotin labeled oligomers⁷³. In the GP 5/6 system, an amplicon of about 140 nucleotides is produced from a region of L1 that overlaps with MY and subsequently separated by gel electrophoresis, blotted onto filters and hybridized with radioactively labeled probes. Both methods are fairly equivalent for in-vitro use, but the GP primers are better for amplification of targets from paraffin-embedded sections, because the longer MY amplicons are not synthesized as efficiently because of formalin cross-linking in the tissue.

The use of type-specific primer systems is an alternative approach for the detection of HPV. This system is of particular use when the determination of a single genotype of HPV is of interest. Additionally, these systems provide an excellent means of confirming results generated by consensus PCR or other methods of HPV DNA detection.

1.2.4 PAPILLOMAVIRUSES AS CAUSATIVE AGENTS IN CERVICAL NEOPLASIA

Genital HPV infections are highly prevalent (20 - 80%) in sexually active age groups⁷⁴⁻⁷⁵. The causative role of HPV in the induction of condylomata has been proven by experimental transmission from person-to-person and in animal model systems. The

etiologic role of HPV in intraepithelial neoplasias was demonstrated by observations of naturally occurring transmission between sexual partners in whom histologically similar lesions developed harbouring the same HPV type⁷⁶. Transfection of human keratinocytes with HPV 16 induces histologic features of intraepithelial neoplasia when cells are grown in organotypic cultures allowing the formation of stratified epithelium⁷⁷. The histology resembles CIN I at the beginning and corresponds to carcinoma in situ after several in vitro passages.

Using PCR-based and other HPV detection techniques, many cross sectional studies have been performed to study the prevalence of HPV in women with normal and abnormal cervical smears. In women with normal cervical smears, HPV prevalence rates varying from 1.5% to over 30% have been reported^{74,78}. Some studies may have suffered from insensitive HPV detection and PCR contamination making comparisons of the different studies difficult. However, prospective studies of women with initially negative cytologic tests showed that the cumulative incidence of CIN II at 2 years was 28% among HPV positive women compared with 3% among HPV negative women⁷⁹. Infection with either HPV 16 or 18 is associated with a relative risk of 11 for development of CIN.

The magnitude of the association between HPV infection and the risk of cervical neoplasia have been examined by different groups. Pooling of data based on Woolf's technique⁸⁰ leads to a combined RR for CIN from all non-PCR studies of 10.3 (95% CI, 6.9 - 15.3), whereas the RR from PCR studies was 19.8 (95% CI, 15.2 - 25.8). The difference between pooled estimates is more pronounced for studies of invasive carcinomas with RR of 3.7 (95% CI, 3.1 - 4.6) and 34.5 (95% CI, 21.5 - 55.4) for non-PCR and PCR studies, respectively. These data place HPV infection as the strongest risk factor for cervical cancer with a magnitude of association that is greater than that for the association between smoking and lung cancer and is second only to the association between the chronic

carrier state of hepatitis B infection and liver cancer, causal relations in cancer that are no longer challenged⁸¹. In addition, recent evidence from a large international study indicates that meticulous testing by PCR of nonfixed specimens of cervical carcinomas results in positivity rates of 95%⁸². A consensus panel of the World Health Organization's International Agency for Research on Cancer (IARC) has concluded that there is now compelling evidence both from biologic and from epidemiologic standpoints to consider that HPV infection leads to cervical cancer⁸³⁻⁸⁵.

Cervical HPV infection detected by DNA hybridization techniques is found in 15 - 40% of sexually active women^{74,85-86}. Most of these infections are transient, and only a small proportion of women tend to harbor the same HPV type on a persistent basis⁸⁷⁻⁸⁹. Prospective epidemiologic studies have indicated that the risk of subsequent cervical neoplasia seems to be proportional to the number of specimens testing positive for HPV⁷⁹. Little is known about risk determinants for persistent HPV infection. The risk of HPV infection seems to be independently influenced by other variables such as parity, oral contraceptive use, and smoking⁹⁰.

1.2.5 MOLECULAR MECHANISMS OF HPV IN CERVICAL ONCOGENESIS

HPV E6 and E7 are small proteins that show some similarities to each other. It has been proposed that they arise following amplification and divergence of a 33 amino acid peptide. The main feature that the two share is a series of Cys-X-X-Cys motifs, which occur four times in E6 and twice in E7, and are thought to play a role in zinc binding by both proteins⁹¹⁻⁹².

1.2.5.1 The E7 Gene

The E7 oncoprotein is a 98 amino-acid phosphoprotein localized to the cell nucleus within the nuclear matrix⁹³. It possesses transforming, immortalizing and *trans* activating

properties and is phosphorylated on serine residues⁹⁴. The amino-terminal 37 amino-acids bear significant sequence homology to conserved domains 1 and 2 (CD1 and CD2) of the Adenovirus 5 E1a oncoprotein as well as to a region of the SV40 large T oncoprotein. CD1 and CD2 have been shown to have several important biological functions such as cooperation with the ras oncogene in transformation assays, stimulation of DNA synthesis, as well as possessing binding sites for cellular proteins which may be important for E1a mediated transformation. Recent experimental evidence has shown that like E1a and SV40 large T antigen, HPV 16 and 18 E7 proteins bind to the retinoblastoma gene product, Rb⁹⁵⁻⁹⁶. The Rb binding domain has been localized to the region of homology with CD2 of the adenovirus E1a protein⁹⁶ and mutations in this domain eliminated Rb binding⁹⁶⁻⁹⁸. This region consists of a stretch of 17 amino acids⁹⁹.

The Rb gene consists of 27 exons spanning 200 kilobases of chromosomal DNA (band 13q14). The associated mRNA encodes a nuclear phosphoprotein with M.W. of 105-110Kda. It is expressed throughout the cell cycle, and it is found in the non-phosphorylated and phosphorylated forms that are specific for certain phases of the cell cycle¹⁰⁰. In the non-phosphorylated state, it acts to restrict cell proliferation, partly by binding to the transcription factor E2F¹⁰¹. E2F is capable of transactivating several genes expressed during the S phase of the cell cycle. E2F-pRb complexes can be identified primarily in extracts of cells at the G1 phase of the cell cycle¹⁰¹. In this complex, pRb is unable to activate promoters which are important positive signals for growth such as *c-myc* and *n-myc*. In the phosphorylated state (G2/S), the control of Rb on cell growth is released¹⁰⁰. Regulation of the phosphorylation is mediated in part through TGF- β 1 probably by blocking phosphorylation of Rb protein¹⁰².

HPV16 E7 binds preferentially to the under-phosphorylated form of Rb and releases E2F from the Rb complex⁹⁸. Furthermore, the HPV E7 gene product associates with the E2F-

cyclin A complex¹⁰³. The complex consists of cellular proteins E2F, p107, cyclin A and cdk 2, all of which are important in the regulation of cell growth at different stages of the cell cycle. The released E2F will activate the expression of cell-cycle regulated genes such as *c-myc*, thymidine kinase, and DNA polymerase alpha, required for entry into the S-phase of the cell cycle. The functional significance of the E7-pRB interaction is underlined by the fact that the E7 proteins of low risk genital HPV types 6 and 11 bind with much lower affinity than the E7 proteins of HPV 16 and 18^{96,104-105}.

The E7 oncoprotein also has another biochemical function which it shares with an area of structural homology to E1a in the carboxy terminal region of CD2. Aminoacids 31 to 37 represent a substrate for casein kinase II (CKII), which phosphorylates serine 31 and 32⁹⁷. Replacement of the 2 serines by non-phosphorylatable amino acids lead to a reduction in transforming activity and abolished phosphorylation, but not Rb binding. CKII has been implicated in the regulation of RNA and protein synthesis as well as DNA metabolism by phosphorylating the enzymes and proteins mediating these processes. It also mediates the phosphorylation of *c-myc* encoded proteins suggesting that it may be involved in cell cycle regulation.

1.2.5.2 The E6 Gene

The E6 protein of HPV has been shown to possess various transforming and immortalizing activities, the most important of which seems to be the ability to co-operate with HPV 16 E7 in the efficient immortalization of primary human epithelial cells^{58,106}. The protein consists of approximately 150 amino acids which are believed to form 2 zinc binding fingers. The base of each finger contains four cysteines (Cys) in two pairs of the motif Cys-X-X-Cys, where X varies among the viruses. These E6 fingers comprise 29-30 amino acids and have been shown to specifically bind zinc in an in vitro binding assay¹⁰⁷.

The oncogenic activity of the E6 proteins of the high risk HPVs has recently been correlated with their ability to interact and inactivate the cellular p53 protein¹⁰⁸⁻¹⁰⁹. The documented effects of wild type p53 on cell proliferation include regulation of the transition from G1 to S phase of the cell cycle¹¹⁰⁻¹¹² and a role in determining cell death through apoptosis. p53 also appears to function normally as a G1-S checkpoint control for DNA damage¹¹³⁻¹¹⁴. Thus normal p53 may function as a 'molecular policeman' monitoring the integrity of the genome¹¹⁴. Removal of policing activities of p53 allows for continuous cycling of cells and the more rapid appearance of chromosomal abnormalities.

E6 binding of p53 leads to an increased rate of p53 degradation by a ubiquitin-directed system¹⁰⁹. The enhancement of p53 degradation has been shown to be mediated only by E6 proteins of 'high risk' HPV types. Crook et al¹¹⁵ have shown that a C-terminal region of E6 is involved in the binding of p53 while a region in the N-terminus is involved in degradation. It would appear that all genital HPV E6 proteins bind p53 but only high risk viruses have the ability to bind with high affinity. The E6 protein targets all quaternary forms of wild-type p53, while mutant p53 proteins are variably resistant to E6 mediated regulation and this correlates with PAb 1620 reactivity¹¹⁶. It appears that the PAb 1620+ conformation is important for recognition of p53 by E6 but is not the actual target for degradation. The function of p53 seems to be dependent on a conformationally flexible domain encompassing about 150 residues in the central portion of the protein.

The enzymatic reactions involved in the ubiquitination of proteins are well characterized. Ubiquitin is a 76 amino acid protein which is found in all eukaryotic organisms. The E1 ubiquitin-activating enzyme stimulates the ATP-dependent formation of a high energy thioester between the carboxyl group of the last amino acid of ubiquitin and a thiol group of a cysteine residue of the E1 protein. The E1 protein then transfers the activated ubiquitin to a cysteine of an E2 ubiquitin conjugating enzyme, with retention of a high energy thioester

bond. The E2 proteins usually require an E3 ubiquitin ligase protein to specify proteins that are to be multiubiquitinated. In the case of HPV 16 and 18 E6 interaction with p53, this ligase is a 100KDa cellular protein, called E6 associated protein (E6-AP). Neither E6 nor E6-AP alone can stably associate with p53. The 3 functional domains of E6-AP which are important for the association has been characterized to an 18 amino acid region from amino acid 391 to 408 for binding, a 502 amino acid region from 280 to 781 for the E6 dependent association of E6-AP with p53, and the C terminal 84 amino acids for the E6 and E6-AP dependent ubiquitination of p53¹¹⁷.

Several studies of p53 sequences in tumours and tumour cell lines have shown that while HPV- negative tumours express mutant p53 sequences, only wild type p53 is detected in HPV- positive cancers¹¹⁵⁻¹¹⁸. However, Kessis et al¹¹⁹ provided recent evidence that HPV infection and p53 mutations are not mutually exclusive and that some HPV negative carcinomas may arise from a pathway independent of p53 inactivation. Indeed, an overview of data from several studies suggested that overall, the rate of p53 mutations in HPV positive carcinoma is only 3%; whereas in HPV negative tumours , it is 15%¹²⁰.

1.2.5.3 The E5 and E2 Genes

Both HPV 6 and HPV 16-E5 proteins form complexes with the p16 component of the vacuolar ATPase, which serves the acidification of intracellular compartments¹²¹. E5 of HPV 6 also associates with the receptors for platelet-derived growth factor (PDGFR) and epidermal growth factor (EGFR)¹²². The HPV 16 E5 stimulates the transforming activity of EGFR by enhancing growth factor mediated signal transduction to the nucleus^{54,123}.

The lack of immortalizing activity of a HPV 16 variant from normal human cervical keratinocytes with a mutation in the E2 gene may point to a fourth viral oncogene¹²⁴. It is unlikely that E2 and E5 have a major role in the maintenance of the malignant phenotype of

cancer cells because they are frequently destroyed by integration of the viral genome into cellular DNA.

1.2.5.4 Physical State of Viral DNA

The integration of HPV 16 or 18 DNA into the genome of cancer cells appear to be a potentially important step in tumour progression. Opening of the viral genome at the time of integration frequently disrupts the regulator genes E1 and E2 and engineered mutants in these genes revealed increased transformation efficiency *in vitro*⁴¹. HPV 18 DNA is integrated in most cancers. However, a substantial proportion of HPV 16-positive tumours and one cancer derived cell line revealed only episomal viral DNA^{122,125}. This demonstrates that integration is not a necessary prerequisite for tumour progression.

A specific mechanism for upregulation of E6/E7 expression has recently been shown to operate in carcinomas containing only episomal HPV 16 DNA. The promoter of E6/E7 of the wild type HPV 16 genome is downregulated by a silencer element in the viral control region, which depends on interactions with cellular transcriptional regulator yin-yang 1 (YY1) with four binding sites¹²⁶. Analysis of six cancers carrying exclusively extrachromosomal HPV 16 DNA revealed deletions affecting one to four YY1-binding sites¹²⁶⁻¹²⁷. All of these mutations resulted in a four to six fold increased activity of the E6/E7 promoter suggesting that deletion or mutation in the target sequences for the cellular repressor represents a repeatedly used strategy of HPV 16 to escape from cellular control. A deletion of 38bp from integrated HPV 16 DNA in the cervical carcinoma cell line SiHa¹²⁸ removes one YY-1 binding site indicating that inactivation of YY1 target sequences is not restricted to episomal HPV DNA in cancers.

1.2.6 Summary of evidence for the role of HPV in cervical oncogenesis

1. The incidence of HPV-16 DNA in CIN lesions increases proportionately with their severity.
2. PCR-detectable HPV-16 DNA occurs in more than 50-90% of cervical cancer biopsies.
3. HPV-16 DNA in cervical cancers is often integrated into host DNA.
4. HPV-16 DNA is retained in continuous cell lines from cervical cancers.
5. HPV-16 DNA can transform and immortalize human keratinocytes in vitro, whereas non-cancer associated HPVs do not .
6. HPV-16 E6 and E7 proteins inactivate endogenous tumour suppressor proteins p53 and pRb, respectively .

1.3 THE HUMAN IMMUNE SYSTEM

The human immune system is equipped with several different functional cell types which are involved in the identification and subsequent destruction of infectious agents. A division can be made between the specific and the non-specific immune response. The latter is represented by natural killer cells (NKs) and macrophages, which do not require specific priming for lytic functions and lack immunological memory. Macrophages can be enhanced in their lytic activity by cytokines such as γ -IFN, IL-2 and M-CSF. Activated macrophages can kill target cells by production of cytotoxic products, including TNF- α , or by mediating antibody-dependent cell-mediated cytotoxicity (ADCC), using their Fc-receptor. NK cells may recognize and kill target cells which lack MHC class I molecules on their cell surface, according to the hypothesis of 'missing self' recognition¹²⁹. In addition, NK cells can also engage via ADCC using their Fc receptor.

The two main categories of specific immune response are the humoral immune response, making use of antibodies, and the cellular immune response, mediated directly by T cells.

1.3.1 ACTIVATION OF THE IMMUNE RESPONSE

Upon invasion of a host, a pathogen may reside either in the extracellular space or within a cell's interior. Cell associated receptors can readily detect extracellular material but cannot directly recognize ligand separated from the receptor by a lipid bilayer. The detection task is further complicated by the existence of two distinct subcompartments for intracellular pathogen residence within a cell: the cytoplasm and membrane-bound endocytic organelles.

A central role is played by the 'professional' antigen presenting cell (APC) in the onset of both the cellular and humoral immune response (Fig 1.2). These cells, including the Langerhans' cells and interdigitating cells¹³⁰, retain the ability to take up antigenic proteins, degrade these in the endocytic route and present small protein fragments or peptides at their cell surface to lymphocytes. Also B cells and monocytes/macrophages can process and present antigens in a similar fashion. The peptides are presented at the cell surface by major histocompatibility complex class II (HLA class II) molecules, after which the specific HLA/peptide interaction can be recognized by the T cell receptor (TcR) of CD4+ helper cells (Th). Recognition of the antigen as non-self leads to the activation of the specific Th cell, which then proliferates and starts producing different lymphokines, that stimulate various other immune cells. Activated Th cells have occasionally been found to exert cytolytic functions as well¹³¹.

Two subsets of CD4+ cells, designated Th1 and Th2 have been recognized based on the cytokines they express as well as functional properties¹³²⁻¹³³. Th1 clones produce IL-2, γ -IFN and TNF- β , thereby providing help to cell mediated effector responses. Th2 clones secrete IL-3, IL-4, IL-5, IL-6 and IL-10, which stimulates B cells to produce antibodies¹³³. The conditions that dictate which Th clone develops after antigenic stimulation are not fully understood and may be determined, at least in part by the invading virus. The cytokines produced by one Th clone can inhibit cytokine production by the other

Th clone¹³⁴. Also transforming growth factor- β (TGF- β) inhibits IL-4 and -5 production by Th, while g-IFN and IL-2 production remain unaffected, suggesting that in particular, Th1 stimulation is promoted¹³⁵. Furthermore, co-stimulatory signals from the APC may dictate which Th clone develops, since the requirements of costimulatory signals is different for Th1 and Th2 cell¹³⁶.

1.3.2 HUMORAL IMMUNE RESPONSE

Generally, B-cell responses require help from T cells. A B-cell recognizes a determinant on a native antigen via its membrane bound immunoglobulin (ig). The antigen is internalized, processed and the resulting peptides are presented on the cell surface by HLA class II. The subsequent recognition by the TcR of a CD4+ Th cell leads to activation of the B cell, either as a result of direct contact with the TcR (cognate interaction) or by lymphokine production by the activated Th cell. Activation of B cells leads to clonal expansion and differentiation into antibody producing plasma cells¹³⁷. Antibodies recognize intact protein structures , which allows them to bind and recognize free viral particles. In addition, by binding to structures present at the cell surface of host cells, antibodies can effect complement fixation ('classical' complement fixation pathway), promoting phagocytosis and damage to plasma membranes via the membrane attack complex (MAC). Furthermore, antibodies can enhance the effector functions of the non-specific cellular response via antibody-dependent cell-mediated cytotoxicity (ADCC).

1.3.3 CELLULAR IMMUNE RESPONSE

Cellular immune response involves two types of reactions mediated by different T cell subsets: delayed type hypersensitivity (DTH), initiated by CD4+ T cells and T cell mediated cytotoxicity mediated by CD8+ T cells (cytotoxic T-lymphocytes, CTL). Both require antigen specific priming and retain immunological memory.

In DTH, sensitized Th cells are activated by antigen, presented by APC and the resulting cytokine production recruits and activates lymphocytes and macrophages capable of inflicting local tissue damage. In T cell mediated cytotoxicity, CTLs constitute the main effector targeted towards endogenous antigens such as viral proteins. Also the CTL response is dependent on cytokine production by the Th cells, mainly IL-2 produced by Th1. Since the present work concerns immune response to the human papillomavirus, the CTL-mediated cytolytic pathway is discussed in more depth below.

1.3.3.1 T cell mediated cytotoxicity

CTLs are CD8+ T cells that recognize antigenic peptides presented by HLA class I¹³⁸. Interaction of the TcR with MHC-I/peptide leads to the activation of CTL and expression of its IL-2 receptor. Subsequent clonal expansion is mostly dependent on IL-2 production by the activated Th1¹³⁹⁻¹⁴⁰. This results in an increased number of antigen specific CTL that express cytolytic agents, packaged in cytosolic granules. The contents of such granules include serine proteases or granzymes¹⁴¹, the pore-forming macromolecular complex perforin or cytolyisin¹⁴² and the calcium-binding protein cal-reticulin¹⁴³.

When the activated granzyme expressing CTL engages with its target cell, the TcR is triggered by the appropriate MHC-I/peptide combination. This activates protein kinase-C (PKC), resulting in phosphorylation of lymphocyte function-associated antigen-1 (LFA-1)¹⁴⁴. As a consequence, the affinity of LFA-1 for its ligand intercellular adhesion molecule-1 (ICAM-1), present on the target cell is increased¹⁴⁵. Furthermore, phosphorylated LFA-1 associates with the cytoskeletal protein talin¹⁴⁶, which stabilizes LFA-1 expression at the cell surface. The enhanced LFA-1/ICAM-1 interaction further consolidates the CTL-target cell contact.

In addition, engagement of the TcR results in a rapid reorientation of granules in the CTL towards the target cell¹⁴⁷. This probably involves reorganization of the microtubuli organization centre (MTOC) and the golgi apparatus¹⁴⁶, which promotes the intracellular flow of vesicles towards the CTL-target cell contact site. Furthermore, the association of LFA-1 with talin stabilizes talin clusters under the CTL membrane in the proximity of the CTL-target cell contact area, which promotes fusion of secretory vesicles with the CTL membrane. The combined action of the MTOC/Golgi reorientation, and the talin/LFA-1 association results in exocytosis of the cytolytic granules into the luminal cleft between the CTL and the target cell¹⁴⁷. Either through the pore-forming function of perforin¹⁴⁸, or by specific adherence and subsequent endocytosis of the granule¹⁴⁹, the cytolytic components are delivered to the target cell. A number of other cytotoxicity pathways involving CTLs have been described. These include secretion of lymphokines such as TNF- α and TNF- β by CTL which are cytotoxic to some cells¹⁵⁰.

1.4 THE HLA COMPLEX

The HLA gene complex is found on the short arm of chromosome 6 in the 6p21.31 to 6p.33 region where it encompasses approximately 3,500 to 4,000 Kilobases of DNA (Fig 1.3). The HLA class I region spans approximately 1600 to 2000 Kb and contains genes encoding the classic class I antigens: HLA-A, HLA-B, and HLA-C as well as the three non-HLA-A, B, C class I genes: HLA-E, HLA-F, and HLA-G. The HLA D region contains the genes for HLA-DR, DQ, DP, DN, and DO and spans 1000 to 1200 Kb of DNA. Between the class I and class II regions lies the class III region, which contains at least 35 genes including complement factors (C2, C4 and Bf), steroid 21-hydroxylase (CYP21), heat shock protein 70, opposite strand gene (OSG), and tumor necrosis factor alpha and beta. Recently, genes encoding molecules involved in antigen processing and assembly of class I molecules as subunits of a large multifunctional protease (LMP) and as

a membrane transporter associated with antigen processing (TAP) were mapped to the class II region¹⁵¹.

The HLA complex covers a relatively small segment of the chromosome corresponding to approximately 2 centimorgans. This means that genetic recombination occurs very infrequently and the complex can be considered as a single genetic unit. The genetic unit composed of HLA alleles present on the HLA-A, HLA-B, HLA-C, and HLA-D loci on each of the two homologous chromosome 6 is called an HLA *haplotype*. The two haplotypes present in each individual constitute the HLA *genotype*. The gene products of each of the class I and class II loci are co-dominantly expressed as cell surface antigens. This means that each individual expresses two HLA-A antigens, two HLA-B antigens, two HLA-C antigens, and two sets of HLA-D gene products. These HLA antigens constitute an individual's *phenotype*. An important characteristic of the HLA gene complex is the existence of *linkage disequilibrium* between the alleles of the loci. In a random mating population at Hardy-Weinberg equilibrium, the joint frequency of 2 alleles from 2 different loci will be the product of their individual gene frequencies. If the observed value of the joint frequency is significantly different from the expected frequency, the 2 alleles are said to be in linkage disequilibrium.

Most expressed HLA genes exhibit a remarkable degree of allelic polymorphism. This is the occurrence in the population of two or more genetically determined forms in such frequencies that the rarest forms could not be maintained by mutation alone. The molecular genetic basis for polymorphisms of HLA class I and class II alleles is due to differences in nucleotide sequences within the coding regions of the individual HLA genes. HLA polymorphism has several unique features: most have many alleles, no allele dominates in frequency, and alleles differ by many amino acid substitutions. Although the reasons for this extensive genetic polymorphism are currently unknown, there are two dominant

theories, namely, retention of ancestral polymorphisms and hypermutational diversification¹⁵²⁻¹⁵³. It is evident that most major MHC allelic types diverged prior to the origin of the species in which they are found based on sequence data from rodent and primate MHC genes^{152,154-155}. The rate of amino acid altering substitutions exceeds that of silent substitutions in codons of contact amino acids in the antigen binding site of MHC class I and Class II molecules, indicating that selection operates directly on the antigen binding site¹⁵⁶. The high degree of polymorphism, long persistence of alleles, low frequency of homozygotes, and high rate of replacement substitutions is probably best explained by overdominant selection.

1.4.1 CLASS I AND CLASS II HLA MOLECULES

The main biological function of HLA molecules is to bind peptide fragments of processed protein antigens and present them to T cells. Class I molecules consist of two common subunits; a polymorphic 45-kDa heavy chain glycoprotein that is non-covalently associated with a conserved 12 k-Da β_2 -microglobulin (β_2 M) light chain¹⁵⁷⁻¹⁵⁸. Class I molecules are expressed on virtually all nucleated cells¹⁵⁹. The class I heterodimer is expressed as a transmembrane complex at the cell surface with three N-terminal heavy chain domains called α -1, α -2, and α -3, extending outward from the membrane. The heavy chain- β_2 M complex bound with its antigenic peptide is anchored by a single transmembrane segment on the heavy chain that is followed by a short intracytoplasmic sequence of variable length. The membrane proximal external domain, α -3, folds in a manner similar to that of an immunoglobulin domain and has several extensive contact with the β_2 M light chain. The structure of several class I molecules as determined by X-ray crystallography (Fig 1.4) reveal that the two most N-terminal domains fold as a unit to form a prominent groove on the top face of the molecule^{138,160-161}. Two parallel α helices and eight antiparallel β sheets comprise the walls and base of the groove, respectively. The groove was found to be of dimensions appropriate to accommodate short peptides (8 - 10 residues).

Class II MHC proteins consist of a 33 KDa alpha chain that is noncovalently associated with a 28 KDa beta chain¹⁶²⁻¹⁶³. Both chains are glycosylated transmembrane proteins, and each consists of two extracellular domains ($\alpha 1$ and $\alpha 2$; and $\beta 1$ and $\beta 2$), a hydrophobic domain, and a short cytoplasmic segment. Class II molecules are found on B cells, activated T cells, macrophages, monocytes, dendritic cells and endothelium, except under the influence of the cytokine gamma interferon, which induces class II expression on diverse cell types. As determined by X-ray crystallography, the N-terminal α -1 and β -1 domains of the class II subunits fold in a manner analogous to that observed on class I (Fig 1.4) and form a groove similar in overall structure to that observed on class I, with the notable exception that unlike class I, the ends of the class II groove are open¹⁶⁴.

One of the most important features of HLA molecules is their ability to form stable complexes with several different peptide sequences. This enormous binding capacity arises from hydrogen bond interaction between conserved HLA residues and the peptide main chain, thus providing sequence-independent affinity for peptide ligands^{161,165-166}. HLA-peptide interaction also involves polymorphic residues in the HLA molecule and specific side chains of the peptide. Some of the peptide side chains contact residues within the HLA cleft and increase the overall binding affinity and specificity of the associated peptides (anchor residues)¹⁶⁷⁻¹⁷⁰; others interfere with residues of the HLA cleft and reduce binding (inhibitory residues)¹⁷¹⁻¹⁷³. These sequence-dependent interactions are due to "pockets" which stud the grooves of both HLA classes¹⁶⁴ and the side chains of polymorphic residues contribute to the walls and floors of these pockets. Thus, the distinct chemical and size characteristics of these pockets in different MHC molecules result in strong preferences for interacting with certain amino acids side chains. For example, a negatively charged side chain in one HLA molecule may preferentially interact with positively charged peptide residues, whereas a positively charged side chain in another HLA molecule may only bind to negatively charged peptide residues. The residues that fit optimally (anchor residues) into

these pockets occur with high frequency in specific positions in peptides associating tightly with particular HLA class I or class II molecules¹⁶⁹⁻¹⁷⁰ and most HLA molecules require two to three anchors in a peptide for optimal binding. For any given HLA allele, the anchor positions are at fixed distances from one another and involve only a few specific amino acids. They can therefore be described by simple motifs, which is proving to be a useful way of predicting which segments of a protein may be efficiently presented by a given HLA allele. Once they have been produced and transported to the plasma membrane, peptide-MHC molecule complexes function by interacting with clonally distributed receptors of T lymphocytes.

In contrast to peptides associated with HLA class I, those associated with HLA class II are commonly presented as nested sets and are typically 10-34 residues in length¹⁷⁴⁻¹⁷⁵. The term 'nested' set refers to a family of peptides sharing a common core sequence with extensions/truncations at either the N- or C- terminal ends. The ability of peptides to vary considerably in length is consistent with the open ended structure of the HLA class II binding groove¹⁶⁴. Most peptides bound to HLA class II have either an aliphatic or aromatic residue near the N-terminus, which presumably fits into the 'hydrophobic' pocket formed by $\alpha 22$, $\alpha 26$, $\alpha 31$, $\alpha 54$, $\beta 85$ and $\beta 86$ residues of the HLA class II molecule. This pocket seems to be capable of accommodating many different hydrophobic residues (e.g Ile, Leu, Met, Val, Phe, Tyr or Trp)^{170,172}. The fact that many different hydrophobic residues are accepted in this position, combined with the occurrence of substantial variations in peptide length, has hindered the identification of other anchor positions by simple sequence alignment.

1.4.1.1 Viral Antigen Processing and Presentation: Class I Pathway

Both infectious and noninfectious forms of viral antigen can enter the endocytic pathway of a professional APC to be processed and presented, in the context of HLA class II

molecules, to CD4+ cells. However, infection with live virus is a requirement for induction of class I-restricted CD8+ T cell responses^{176,177}. This difference results from the distinct intracellular location of processing activities for HLA class I and class II antigen presentation. Viral epitopes presented by class I molecules are derived from viral proteins synthesized *de novo* in an infected cell, whereas viral antigens that enter the endosomal/lysosomal compartments have been specifically routed to these compartments, usually after capture from an extracellular location.

The cytosolic proteolytic processing enzymes required for the generation of class I-presented viral peptide fragment have not been positively identified. However, proteasomes and a larger ubiquitin-dependent complex are attractive candidates for generating peptides from larger proteins because of their broad specificity, ability to cleave on the carboxyl side of hydrophobic, basic, or acidic residues, and the demonstration that proteasomes can process proteins into oligomers within an appropriate length range for class I binding without further degradation to single amino acids¹⁷⁸. The finding that two proteasome subunits, LMP-2 and LMP-7 (for low molecular mass polypeptide), are encoded in the HLA class II region further implicated the involvement of proteasomes in antigenic processing¹⁷⁹. Recently, it was demonstrated that the MHC encoded LMP gene products specifically alter the peptidase activity of the proteasome to favor cleavages that result in peptides possessing basic residues on their C-termini¹⁸⁰⁻¹⁸¹ which is necessary to anchor peptide binding in class I grooves.

The intracellular association of appropriate octamer or nonamer peptides with class I heavy chains is essential for stable assembly and transport of peptide-loaded class I complexes to the cell surface¹⁸²⁻¹⁸³. Peptides bind newly synthesized and translocated class I molecules in the endoplasmic reticulum (ER)¹⁸⁴⁻¹⁸⁵. The empty class I molecule may temporarily be stabilized in ER by complexing with p88 (also termed calnexin), a chaperone-like

molecule¹⁸⁶⁻¹⁸⁷. Binding of peptide results in the release of p88¹⁸⁷. The genes responsible for peptide translocation over the ER membrane have been identified¹⁸⁸⁻¹⁸⁹, and are now designated the transporter for antigen presentation (TAP) 1 and 2 genes, previously known as peptide supply factor (PSF) or Really Interesting New Gene (RING-4 and -11). They belong to the superfamily of ABC transporters, displaying properties like an ATP-binding cassette.

1.4.1.2 Viral Antigen Processing and Presentation: Class II Pathway

HLA class II molecules present peptide fragments derived from exogenous protein antigens, including structural components of virus particles or secreted viral proteins, to CD4+ cells. Exogenous viral antigens are taken up into endosomal compartments, cleaved into short peptides that associate with class II molecules targeted to this compartment, and then the peptide-MHC II complexes are routed to the cell surface for T cell recognition^{137,190-191}.

On translocation to the ER, HLA class II α and β chains rapidly associate with one another together with a third, nonpolymorphic or invariant (Ii) chain¹⁹². The Ii chain is a type II transmembrane protein, with the amino terminus extending into the cytosol and the C terminus residing in the lumen of the ER¹⁹³. The Ii chain has been demonstrated to prevent exogenous peptides from binding the associated HLA class II¹⁹⁴⁻¹⁹⁵. The α/β -Ii trimeric complex is transported through the Golgi to the trans-Golgi reticulum (TG), where the cytoplasmic domain of the Ii chain targets the class II molecule into the endocytic compartments¹⁹⁶. Subsequently, the Ii chain is degraded by proteases in the acidic environment of the endosomes, which renders the class II molecule free for peptide binding¹⁹⁷.

Proteolytic cleavage of the li chain yields large fragments termed LIP and SLIP¹⁹⁸⁻¹⁹⁹ as well as a set of nested invariant chain fragments termed CLIP²⁰⁰. The CLIP epitope resides in the class II binding groove potentially to prevent peptide loading in early biosynthetic compartments. Release of these invariant chain fragments allows antigenic peptides to bind to class II proteins. Although the spontaneous dissociation of CLIP from class II molecules is observed at low pH²⁰¹⁻²⁰², a novel MHC heterodimer DM has been identified that enzymatically catalyzes rapid CLIP dissociation²⁰³⁻²⁰⁴. The ability of HLA-DM to release invariant chain fragments has led to the proposal that DM functions as a peptide editor and triggers the dissociation of unstable peptides from class II proteins²⁰⁵. In this way, DM may catalyze the release of suboptimal peptides from class II proteins and directly influence epitope selection. However, alternate mechanisms within APC may also control peptide loading, including antigen trafficking, sites of processing, and the protease content of the APC.

Where in the endocytic route the class II molecule picks up peptide is not clear, and may differ per cell type and per antigen. Endocytosis of soluble antigens can be accomplished by internalization of surface Ig plus bound antigen (B cells), by absorption of soluble antibody-antigen complexes by Fc-receptors (NK cells and macrophages), or by fluid phase endocytosis. The endocytosed antigen proceeds through the early and late endosomes to lysosomes. The proteolytic enzymes involved in antigen breakdown, such as cathepsin D and E²⁰⁶⁻²⁰⁷, are probably of sufficient concentration in the late endosomal/lysosomal stage¹⁹⁷. Here these peptides may be protected from further breakdown into single amino acids by binding to class II, which makes the late endosomal/lysosomal compartments the most likely site where the class II molecule meets its antigenic peptide. Similar to class I, the stable cell surface expression of HLA class II is enhanced by binding of peptide.

1.4.2 HLA DNA TYPING STRATEGIES

Until recently, HLA typing was dominated by serological and cellular techniques. Within the last 10 years, more powerful DNA-based typing methods have evolved, and these have proven considerably more accurate and reproducible than conventional serological or cellular typing.

Prior to the advent of the polymerase chain reaction, the most widely used DNA-based HLA class II typing method was restriction fragment length polymorphism (RFLP) analysis²⁰⁸. RFLP entails the restriction endonuclease digestion of genomic DNA followed by electrophoretic resolution of the endonucleotic fragments which are denatured in situ and hybridized to a nylon membrane. The membrane is then probed with a homologous labeled cDNA or genomic probes which yield hybridization signals characteristic of various HLA alleles. Although RFLP is considerably more accurate than DR and DQ serotyping, it has certain disadvantages. The technique is technically demanding, takes around 7 days to complete, relies heavily upon linkage disequilibrium between DR and DQ loci for identification of certain alleles, and it does not define allelic variation at the level of the second exon of the gene.

The development of the polymerase chain reaction (PCR)²⁰⁹ allowed the evolution of improved molecular HLA-typing techniques. PCR is used to generate specific amplified stretches of DNA sequences in vitro through repeated cycles of DNA denaturation, annealing of specific primer to a single strand, and nucleotide extension from primer pairs using a DNA polymerase. Currently, most HLA class II typing methods rely on the amplification of the second exon of the polymorphic DRB, DQA, DQB and DPB genes followed by a simplified analysis of allele-specific nucleotide sequences within the hypervariable regions of the exon. The techniques for analyzing polymorphisms in

amplified DNA can be divided into two basic groups: probe hybridization and direct amplicon analysis.

Probe hybridization techniques rely on amplification of a target DNA sequence which is generally immobilized onto a support membrane (known as dot blotting). The initial amplification is normally generic but may be a mosaic of amplifications which when used together amplify all possible alleles of a given locus. The polymorphisms in the immobilized DNA are subsequently detected by using specific single-stranded DNA probes in combination with highly stringent washes to remove non-specifically bound probe²¹⁰. In addition to the use of radioactive isotopes, hybridized probes can be detected by a variety of non-radioactive methods, such as horseradish peroxidase²¹¹ and digoxigenin labelling²¹². The technique became known as PCR-SSOP (PCR followed by sequence specific oligonucleotide probing, also discussed in chapter 2). PCR-SSOP was first applied to histocompatibility testing in HLA DQA1 by Saiki et al (1986). Subsequently, the method was applied to HLA DRB1²¹³⁻²¹⁴, HLA DQB1²¹⁵ and a combination of DR, DQB1, DPA1, and DPB1²¹⁶.

The method is useful for analyzing a large number of samples at once. However, it is a time consuming and expensive method to use to define HLA types in a small number of samples. This led to the development of an alternative strategy, the reverse PCR-SSOP method²¹⁷, where a panel of SSO probes are immobilized on a single membrane by means of poly-T tails, leaving the detection end of the probe free to interact with target DNA²¹¹. Biotin-labeled PCR-amplified target DNA is hybridized with the membrane bound SSO probes. Following stringent washing, the specificity of hybridization is revealed using streptavidin-horse radish peroxidase as the conjugate. This converts a chromogenic substrate into a coloured precipitate²¹⁸. Another modification of PCR-SSOP is PCR-HPA (hybridization protection assay), and is based on nucleotide hybridization utilizing

acridium ester labeled SSO in the liquid phase. As in the case of the PCR-SSO method, the design of the SSO is critical for the accuracy of this technique.

Current PCR-SSOP approaches require lengthy post-PCR steps. This has led to the development of direct amplicon analysis techniques. These methods, while not so efficient for large numbers of samples, are more suitable for rapid limited sample number throughput. These include PCR-RFLP, PCR-SSP, nested PCR-SSP, heteroduplex analysis, and other conformational assays.

The first of these methods, the PCR-RFLP depends on sequence recognition by restriction enzymes. The main advantages of this technique are that sequence variations at different positions can be recognized at once if there are several restriction sites in the region analyzed, it may be performed in less than 5 hours, and it eliminates the requirements for radioisotopes, probes or reporter molecules. However, some of the currently known alleles cannot be easily distinguished because of the unavailability of restriction enzymes recognizing their sequence variations. Furthermore, the identification of some allelic combinations in heterozygous individuals is not possible²¹⁹ or is complicated by incomplete digestion of PCR products.

Another method, allele specific amplification of an allele or group of alleles is based on the fact that PCR cannot be accomplished if the 3' end of primer has a mismatch(es) with a given allele. Newton et al²²⁰ described the detection of a single point mutation using one generic sense primer and two antisense primers: one antisense primer was specific for the "normal" form and was refractory to PCR on "mutant" DNA, and the other antisense primer for the "mutant" was refractory to PCR on "normal" DNA. This was termed the amplification refractory mutation system (ARMS). The technique works because *Taq* polymerase lacks 3' to 5' exonucleotic proof-reading activity. For efficient ARMS

amplification without false priming, the conditions need to be highly stringent. The first comprehensive ARMS HLA typing system was described in 1992 by Olerup and Zetterquist²²¹ for low resolution HLA DRB1 typing, including group specific detection of DRB3 and DRB4 by ARMS using 19 PCR reactions. Olerup and Zetterquist²²¹ renamed the assay PCR-SSP (PCR using sequence-specific primers). Modern PCR-SSP features multiple PCR reactions where each reaction is specific for an allele, or group of alleles. The method requires a large number of primers to detect a specific allele and is therefore used more often to detect groups of alleles. For example, PCR primers, complimentary to conserved flanking sequences of second exons from a group of DRB1 loci, will generate a mixture of PCR products, depending on the DR haplotype. This is useful since it permits dot-blot, reverse dot-blot PCR-SSO and PCR-RFLP typing of the individual alleles simultaneously.

A modification of the PCR-SSP method is the nested PCR-SSP technique first described by Bein et al²²². In this method, the region of interest is amplified in the first step and this amplicon is used instead of genomic DNA for the second sequence-specific amplifications using primers which are internal to the first set of amplification primers. The results obtained by this method are similar to one-step PCR-SSP. The advantages of the nested PCR-SSP over conventional PCR-SSP include the very small amount of DNA required for the former, and the possibility of subtyping highly polymorphic alleles.

PCR-heteroduplex formation or 'DNA crossmatching' is another direct amplicon analysis method. At the end of any PCR cycle, the individual strands may re-anneal with each other to form homoduplexes, or they may re-anneal with an unrelated DNA strand to form a heteroduplex, or they may remain as single stranded structures²²³. These different forms of PCR products have unique conformational structures which may be differentiated by their electrophoretic mobilities in a temperature or denaturing gradient gel. PCR heteroduplex

analysis has never gained popularity for identifying HLA polymorphisms due to the complexity of the gel analysis and the technically challenging conditions. However, it has been used to match individuals for HLA DR and HLA DP by "DNA crossmatching"²²⁴.

The single-stranded conformation polymorphism (SSCP) analysis depends on the fact that single-stranded DNA molecules of differing sequences exhibit conformational changes as a result of intra-strand complementary base pairing²²⁵. The single-stranded products exhibit different mobilities in nondenaturing polyacrylamide gel electrophoresis that can be used to ascertain the genotype of an individual. So far SSCP has been successfully applied to HLA A, DRB1, DQB1, DQA1, DPA1 and DPB1 typing, and HLA-DR4 subtyping. However, like heteroduplex analysis, the complexity of both the technique and the interpretation has prevented the widespread application of this technique.

Finally, HLA typing by direct amplicon analysis can be accomplished by directly sequencing the PCR products (sequence-based typing, SBT). The principles of SBT are that the polymorphic regions of any given allele are amplified by flanking PCR primers. The resulting PCR products are sequenced by one of a variety of methods and are analyzed by computer to ascertain the type. Computer analysis is required because the sequenced product from a heterozygous individual will contain two superimposed sequences that need to be aligned with all previously known sequences in order to be identified and separated. SBT was initially described for HLA DRB1, DQB1 and DQA1 by Santamaria et al²²⁶ and for HLA DPB1 by Rozemuller et al²²⁷. The main drawbacks of SBT are the equipment costs and the time required to fully sequence one individual. Offset against this is the tremendous advantage of having high resolution typing. However, sequencing is not infallible and some sequenced alleles have had to be retracted due to errors, most commonly GC inversions.

1.5 IMMUNE RESPONSE TO HUMAN PAPILLOMAVIRUS

Advances in the understanding of the nature of immune responses to HPV infection has been hampered by three major technological problems. Firstly, there exists no useful animal model of the disease (with the exception of the analogous bovine papillomavirus that is associated with malignancies of the gastrointestinal tract). Secondly, until recently²²⁸, there was no permissive system for propagating the virus in culture in vitro and thirdly, it is difficult to isolate intact genital HPVs from lesions or tumours. Nevertheless, there is now a substantial body of evidence which indicate that humans can mount immunological responses to the genital HPVs. However, the different steps of onset and the efficacy of the immune response is little understood. It is noteworthy that there is no viremia associated with viral replication in lesions and the infected cells are relatively inaccessible to the elements of the immune system that are not associated with the skin. Thus, the principal mediators of the immune reactions to papillomavirus infection are the keratinocytes, intraepithelial lymphocytes, and the dendritic Langerhans cells²²⁹⁻²³¹.

A significant higher prevalence of sera with antibodies to HPV 16-E6 and E-7 have been observed in cervical cancer patients (33% and 23% respectively) compared with healthy controls²³². Antibodies to recombinant proteins and to synthetic peptides corresponding to HPV 16 early and late proteins have been detected in sera from patients with CIN, but the concentrations of these antibodies are generally low²³³. In the case of the rabbit papillomavirus model, carcinogenic progression seems to be accompanied by variable levels of antibody response to the viral proteins, but the antibodies have little or no ability to induce regression²³⁴. Overall, whilst antibodies to early proteins may represent predictive markers of disease progression²³⁵, they are unlikely to confer any protection against subsequent HPV 16 infections. However, neutralizing antibodies to HPV 16 in cervical secretions may prevent reinfections and effective cell mediated immune responses probably explain why the majority of untreated CIN lesions do not progress to malignancy.

There are several lines of evidence indicating that cell mediated immune response is important in the control of papillomavirus infection. There is increased frequency of HPV infection in therapeutically immunosuppressed patients or in immunodeficiencies specifically involving cell mediated immunity²³⁶⁻²³⁷ as well as in patients with HIV infection²³⁸. HPV infections are also found up to 9 times more often in renal transplant recipients compared to the general population²³⁹ and these patients have an increased incidence of CIN lesions²⁴⁰. The presence of HPV 16 or 18 in CIN correlates with a decreased number of Langerhans cells²⁴¹ and decreased numbers of CD4+ cells are observed in CIN lesions²⁴². Furthermore, patients with HPV positive CIN or cancer exhibit decreased natural killer cell activity²⁴³. Regression of warts shows many characteristics of a cell mediated immune response. Histological examination reveals intense mononuclear cell infiltrate in the dermis, and the majority of the infiltrating cells in regressing warts and CIN are CD4+ T cells²⁴⁴. Finally, patients with common variable immunodeficiency (characterized by failure to produce antibodies) appear not to be unduly susceptible to the development of HPV lesions²⁴⁵.

Although antibodies may play a direct role in the clearance of some viruses²⁴⁶, cellular mechanisms are probably the most important tools for the defense against HPV infection. Thus, the mechanisms underlying the impaired production of IgG antibodies may be important in increasing the risk for persistent HPV infection and cancer. Because a class switch from IgM to IgG antibody production in response to a certain antigen is induced by CD4+ regulatory lymphocytes, HLA class II antigens are likely to be involved in the recognition of foreign peptides by these lymphocytes²⁴⁷. CD4+ T lymphocytes are also involved in the function of class I restricted cytotoxic T cells, which are thought to be responsible for lysis of virally infected cells and malignant transformed cells.

The ability to respond to HPV antigens therefore revolves around the capacity of infected cells to effectively present viral epitopes to T cells, and the host immunogenetic background such as the HLA class I or II type is an important parameter in the overall cellular immune response.

1.6 HLA ASSOCIATIONS WITH HUMAN PAPILLOMAVIRUS AND CERVICAL CANCER

The early population studies on HLA association with cervical cancer were from the United States. In the largest of these studies²⁴⁸ in which 253 patients were HLA typed, HLA A11 was significantly decreased in the patients. However, in another report²⁴⁹, deviations in the frequencies of A1, A9 and B12 were noted. The frequency of HLA B5 was increased in the data of Tarpley et al²⁵⁰ on 67 patients while the frequency of HLA B8 was increased in 33 patients studied by Twomey et al²⁵¹. In the combined analysis of data on Caucasian patients from these early studies (391 from the United states and 64 from Germany), HLA B8 was not significantly increased. There are two early reports from South Africa on Indian²⁵² and Black²⁵³ populations. There was no indication of any HLA associations in these studies. Koenig et al²⁵⁴ examined the sera of 89 German patients for antibodies against Herpes Simplex type 1 and type 2 viruses. The titre for type 2 virus was significantly higher in patients positive for HLA B12. Furthermore, in their study of 120 patients, a small but non-significant association between HLA B12 and increased risk of cervical carcinoma was found.

More recently, there have been a renewed interest in HLA association with cervical cancer (Table 1.4). The results have not been consistent probably because of differences in the size and type of population examined and techniques used for the HLA and HPV typing. Wank & Thomssen²⁵⁵ reported on the frequencies of the HLA class II phenotypes in a German population of 66 patients with squamous cell carcinoma of the cervix and

compared with two control groups. The first control group was a local panel of 109 individuals, and the other control group was a caucasian panel of 2,019 individuals from the Ninth International Histocompatibility Workshop. Using serological typing, the frequency of HLA DQW3 antigen in the local panel was 50.4% (Ninth workshop panel, 41.2%), whereas the frequency in the patient group was 87.8%, suggesting that a caucasian female with the HLA DQW3 antigen has a 7.1 times greater chance of developing squamous cell carcinoma compared with females without this antigen ($p = 0.0009$). In addition, there was a weaker association with HLA DR5 which is in linkage disequilibrium with HLA DQW3, but a 12.7 fold decreased relative risk with HLA DR6.

The limitations of this initial report was that serological typing methods were used, the local control panel were not very well defined, and there was no information on the HPV status of patients or controls. Two subsequent reports by the authors²⁵⁶⁻²⁵⁷ using sequence-specific oligonucleotides to define DQ alleles in the same group of patients showed a preferential increase in the frequency of DQB1*0301 (40 of 57 patients; relative risk 8.71, $p = 0.0001$) and *0303 alleles (9 of 57 patients, relative risk 4.5, $p = 0.0012$) in patients with squamous cell carcinoma of the cervix. However, these findings were not correlated with the HPV status of the patients or controls. In addition, these authors also found that 11 of 22 patients with SCC from Tanzania had the HLA DQB1*0602 allele²⁵⁷. The latter study utilized data from South African donors as controls (22.7% frequency of DQB1*0602) and suggested that this antigen may be important ($p = 0.0041$). A full discussion of the limitations of studies by other groups is found in chapter 6.

1.7 SIGNIFICANCE OF HLA ASSOCIATIONS WITH DISEASE: REVERSE IMMUNOGENETICS

The discoveries of HLA associations with certain diseases represent a significant breakthrough in the understanding of the genetics of these diseases. The primary data showing

the associations are increased frequencies of certain HLA antigens in groups of patients as compared with a sample of normal individuals, and usually none of the observed associations are absolute.

The association of HLA with some autoimmune and infectious diseases are well established. These include rheumatoid arthritis, ankylosing spondylitis, Behcet's disease, insulin dependent diabetes mellitus, malaria, schistosomiasis, tuberculosis and hepatitis B. In addition, previous studies have revealed several associations between the HLA system and malignant disease. For instance, Hodgkin's disease-associated with Epstein-Barr virus (EBV)²⁵⁸, thyroid carcinomas²⁵⁹, non-melanoma skin carcinomas associated with HPV²⁶⁰, cutaneous melanoma²⁶¹ and nasopharyngeal carcinoma²⁶².

The process of identifying an HLA association with an infectious disease and then using this information to identify candidate antigens involved in immunity has been termed "reverse immunogenetics". Classical immunogenetics for infectious disease uses an approach in which antigens are identified (often by relatively unrelated criteria such as reactivity with murine monoclonal antibodies) and then immune responses to these are studied. It is then possible to analyze the MHC restriction of the response to these antigens and to map T-cell epitopes. The difficulty with this approach is that there will be immune responses to many antigens of a pathogen, and only some of these may mediate protection. Therefore, it is necessary to assess whether responsiveness to a particular antigen correlates with protection, which is often a difficult task in clinical practice.

"Reverse immunogenetics" has the advantage that its starting point is an observed resistance or susceptibility to a disease in a subset of a population bearing a particular HLA type. The mechanisms of this resistance or susceptibility can then be analyzed by the identification of antigens derived from the pathogen and recognized in the context of the

significant HLA molecules. This approach has been applied to the investigation of HLA B53 mediated resistance to severe malaria and has been used in this thesis.

1.8 APPROACHES TO DEFINING HLA CLASS II BINDING MOTIFS

The characterization of naturally processed peptides bound to HLA class II molecules associated with susceptibility and protection to HPV infection provides an approach towards understanding both antigen processing and peptide binding events *in vivo*. Crystallographic analysis of HLA class II /peptide complexes have shown that class II molecules bind peptides by forming hydrogen bonds to the peptide backbone and by the sequestration of the side chains of the peptide anchor amino acids inside the pockets of the groove of the class II molecule¹⁶⁵. Different class II molecules have different pockets and bind different sets of peptides²⁶³⁻²⁶⁴. The positions and type of residues which anchor a peptide to a particular class II groove determine the peptide binding "motif" for that class II molecule²⁶⁴.

The binding specificity of HLA class II molecules has been analyzed by a variety of methods. Direct binding to class II molecules has been measured using synthetic variants of high affinity binding peptides^{172,265-266}. More recently, *in vitro* binding studies have been employed using libraries of random peptides encoded in the coat protein of M13 bacteriophage¹⁷⁰, or by studying binding of peptide libraries¹⁷³. Another approach is the sequencing of individual peptides and pools of peptides eluted from affinity-purified class II molecules²⁶⁷.

1.8.1 The Use of Large Peptide Repertoires to Identify General HLA Class II motifs

Of all class II isotypes, HLA-DR is the best characterized structurally and functionally. Thus, for class II HLA-DR molecules, motifs have been identified by the analysis of large

peptide pools selected from M13 bacteriophage peptide display libraries^{170,173,268}. This technique is based on the ability of filamentous bacteriophage to display peptides on their outside surface and involves the screening and enrichment of bacteriophage-displaying peptides that bind to a particular protein. By inserting oligonucleotide-encoding peptides known to bind to HLA-DRB1*0101 into the protein-III encoding gene of bacteriophage M13, Hammer et al²⁶⁸ demonstrated that the bacteriophage displaying the appropriate class II ligand can bind specifically to the DR groove. Based on this observation, a large DRB1*0101 binding peptide repertoire was selected from a M13 peptide display library consisting of millions of random peptides. Sequence analysis of the DNA encoding the DRB1*0101-selected peptides led to the identification of peptide positions in which amino acids with similar side chains occurred with increased frequency (anchor residues), thus resulting in a DRB1*0101 peptide-binding motif²⁶⁸. The motif consists of four anchors at relative positions 1, 4, 6 and 9 that are fixed at distances from one another, thus reflecting the architecture of the DRB1*0101 groove in that both the spacing and chemical characteristics of anchor residues correspond to the major pockets 1, 4, 6, and 9 of the HLA-DR cleft.

The screening of bacteriophage libraries has also been applied to other HLA-DR alleles such as DRB1*0401 and DRB1*1101¹⁷⁰. The results show the presence of conserved anchor residues, i.e., anchors found in each of the HLA-DR selected peptide pools, as well as allele specific anchor residues. For example, most of the HLA DRB1*0101, DRB1*0401, and DRB1*1101 selected peptide pools were found to have aromatic and aliphatic amino acids at positions 1 and 4 respectively, whereas strong allele-specific amino acid preferences were identified at position 6: Ala and Gly for DRB1*0101, Ser and Thr for DRB1*0401, and Arg and Lys for DRB1*1101. These results provided the molecular basis for both the promiscuity and specificity of peptide recognition by HLA-DR

molecules. Further, by varying the conditions used to elute bacteriophage from the class II cleft, it is possible to identify secondary anchors at positions 2, 3, and 7¹⁷³.

General HLA class II motifs can also be identified by the characterizing large endogenous bound peptide pools. The technique was originally developed for the definition of class I motifs¹⁶⁹. In this approach, endogenous class II-bound peptide pools are eluted and subsequently analyzed by Edman sequencing^{267,269}. Because the class II-binding cleft is open at both ends and endogenous peptides are not aligned due to the variable length of class II ligands, pool sequencing approaches with class II-eluted peptides failed to reveal patterns as clear as those of class I ligands. However, pool sequencing combined with the alignment of natural ligands and the consideration of predicted pocket structure resulted in class II motifs similar to the ones obtained by the bacteriophage technology²⁷⁰.

1.8.2 The Use of Single-Substitution Experiments on Naturally Processed Peptides to Identify Specific HLA Class II-Binding Motifs

The effects of single residue substitutions in naturally HLA-bound peptides have been studied to identify residues critical to the interaction of these peptides with HLA class II molecules. For example, in the case of HLA DRB1*0101, the importance of an aromatic residue at relative position 1 was initially found by Ala substitutions of the influenza hemagglutinin (HA) epitope 307-319¹⁶⁸. More extensive truncation and single-residue substitution studies on HA 307-319 or tetanus toxoid 830-843 revealed specific class II binding motifs for DRB1*0401, DRB1*1101, and DRB1*0701^{172,265,271}. Substitution experiments on myelin basic protein peptide 84-102 also revealed differential binding for DRB1*1501 and DRB5*0101²⁷²⁻²⁷³.

Comparison of the results from single-substitution studies with other methods, such as M13-displayed peptide repertoires have confirmed the generality of motifs derived from

single-substitution experiments. Furthermore, only this approach is able to reveal the presence of side chains that interfere with peptide binding. These inhibitory residues are of similar importance for binding with class II molecules as the presence of anchor residues¹⁷¹⁻¹⁷³.

1.8.3 The Use of Quantitative Matrices to Identify HLA Class II motifs

Data from X-ray crystallographic studies, large peptide repertoires and single substitution experiments indicate that peptide side chain effects (anchor, inhibitory, or neutral) seem to depend on the position within a particular peptide frame rather than on neighbouring amino acids. These observations led to the approximation that each amino acid in a peptide sequence contributes to the affinity of the peptide independently of the neighbouring amino acids^{173,274-275}. The determination of the effects of each amino acid at all peptide positions resulted in matrices that define quantitatively HLA class II ligand specificity. DRB1*0401 and DRB1*0101 matrices have been determined using 9- and 13-residue-long²⁷⁵ designer peptides (see also chapter 5). More recently, this approach has been extended to the use of "pocket-specificity" profiles to generate quantitative matrices for many HLA class II alleles²⁷⁶.

1.8 AIMS OF THIS THESIS

Despite the compelling evidence implicating HPV in cervical oncogenesis, the majority of women infected with 'high risk' HPV do not develop cervical intra-epithelial neoplasia (CIN) or cancer. It is clear that competent cell-mediated immune response is required to control HPV infection and prevent the development of CIN or cancer, and this in turn is dependent on proper HLA-mediated antigen presentation. The aims of this thesis are:

1. To examine in detail, the association between HLA-DQ and -DR alleles, the human papillomavirus and premalignant disease of the uterine cervix.

2. To identify susceptibility and protective HLA DQ-DR haplotypes in relation to human papillomavirus and premalignant disease of the cervix.
3. To identify naturally processed peptide sequences bound to susceptibility and protective HLA molecules and use this for motif prediction of HPV 16 L1, L2, E6 and E7 sequences that will bind with high affinity to these HLA molecules. This should lay the basis for future work in evaluating HLA DQ and DR restricted immune responses to HPV infection as well as peptide based vaccine approaches for the prevention and treatment of CIN and cervical cancer.

| Bethesda System | Equivalent Terminology |
|-----------------|--|
| ASCUS | Squamous atypia, Pap class II |
| LSIL | Mild dysplasia, CIN1, Koilocytotic atypia, condylomatous atypia, HPV related changes |
| HSIL | Moderate dysplasia, CIN 2, severe dysplasia, carcinoma in-situ, CIN 3 |

Table 1.1: The Bethesda system of classification of squamous abnormalities compared with other nomenclature

| HPV Genotype | Lesion |
|---|---|
| Cutaneous 1,2,3,4,5,7,8,9,10,12,14,15,17,19,20,21, 22,23,24,25,26,27,28,29,36,37,38,41,46, 47,48,49,50,60,63,65 | Cutaneous warts, flat warts, plantar warts, butcher's warts. Cutaneous plaques and papillomas in patients with Epidermo- dysplasia Verruciformis (EV). Skin carcinomas in renal allograft patients and EV patients. |
| Mucosal 6,11,13,16,18,30,31,32,33,34,35,39,40, 42,43,44,45,51,52,53,54,55,56,57,58,59, 61,62,64,66,67,68 | Laryngeal papillomas, condylomata acuminatum, CIN; vulvar, penile and perianal intraepithelial neoplasia; cervical cancer; vulva, penile, perianal and anal cancer, verrucous carcinoma of vulva and penis, Buschke -Lowenstein tumor. |

Table 1.2: HPV genotypes from cutaneous and mucosal lesions (Walboomers et al., 1994).

| Reading Frame | Function |
|---------------|--------------------------------|
| E1 | DNA replication |
| E2 | Transcription, DNA replication |
| E4 | Cytoskeletal disintegration |
| E5 | Transformation |
| E6 | Transformation |
| E7 | Transformation |
| L1 | Viral capsid |
| L2 | Viral capsid |

Table 1.3: Function of HPV gene products

Table 1.4: Summary of Studies on HLA Associations with HPV, CIN and Cervical Cancer

| Reference | Patients | Controls | Population | HLA Typing Method | HPV Detection | Main Results |
|---------------------------|--|----------------------------------|------------|-------------------|---------------------|--|
| Vandenvelde et al. (1993) | 71 (24 CIN I, 21 CINII, 26 CIN III) | 323 (CIN0, not typed for HPV) | Belgian | PCR-ASO | Done | 1. DQB1*03: RR = 2.647 for HPV associated CIN |
| David et al. (1993) | 50 (5 CIN I, 15 CIN II, 30 CIN III) | 99 (CIN0, blood donors) | British | PCR-SSO | Not done | 1. DQB1*03 : RR = 2.5 for CIN III |
| Mehal et al. (1994) | 66 (27 CIN I, 15 CIN II, 24 CIN III) | 60 | British | PCR-RFLP | PCR | Increased risk of CIN for DQB1*03 |
| Apple et al. (1995) | 128 (55 slight/moderate dysplasia, 73 severe dysplasia / CIS) | 220 (CIN0) | Hispanic | PCR-SSO | PCR | 1. DRB1*0407-DQB1*0301: OR=2.22 2. DRB1*1501-DQB1*0602: OR=3.03 3. DRB1*1501: OR=4.75 4. DRB1*1102-DQA1*0501: OR=0.19 for HPV positive severe dysplasia/CIS. |
| Sanjeevi et al. (1996) | 74 (10 CIN I, 41 CIN II-III, 23 CIN ND) | 164 | Swedish | PCR-SSO | Serology (HPV 6,16) | 1. DQB1*0602: OR=5.67 for HPV 16 seropositive cases/controls. 2. DQB1*0303: OR=2.98 for all cases/control. 3. DQA1*0102-DQB1*0602: OR=6.00 for all cases/controls. 4.DQA1*0501-DQB1*0301: OR=3.00 for seronegative cases/controls. 5. DR15-DQA1*0102-DQB1*0602: OR=6.8 |

| Reference | Patients | Controls | Population | HLA Typing Method | HPV Detection | Main Results |
|------------------------|-------------------------------|-----------------------|------------------|--------------------|---------------|---|
| Helland et al. (1998) | 92 (10 CIN II, 82 CIN III) | 225 (CIN 0) | Norwegian | PCR-SSO | PCR | 1. DQB1*0602: OR=3.2 for HPV positive cases. 2. DQB1*0604: OR=0.1 3. DQA1*0102-DQB1*0601: OR=3.2 for HPV positive cases. |
| Wank & Thomssen (1992) | 66 (SCC) | 109 (local panel) | German | PCR-SSO | Not done | Increased risk of SCC for DQB1*0301 & 0302 |
| Helland et al. (1992) | 213 (SCC) | 181 | Norwegian | PCR-SSO | Not done | 1. DQW3: RR=2.0 |
| Amar et al (1993) | 30 (SCC) | 400 (local panel) | Jewish | PCR-SSO | Not done | No HLA associations |
| Glew et al. (1993) | 65 (SCC) | 857 (organ donors) | British | PCR-SSO & Serology | PCR | No HLA associations |
| Nawa et al. (1994) | 23 (SCC) | Int. workshop | Japanese | PCR-RFLP | PCR | Increased risk of SCC for DQB1*03: p=0.0003 |
| Apple et al. (1994) | 98 (SCC) | 220 (CIN0) | Hispanic | PCR-SSO | PCR | 1.DRB1*1501-DQB1*0602: OR=2.87 OR=4.78 for HPV 16 +ve cases 2.DRB1*0407-DQB1*0302: OR=2.19 3.DR13: OR = 0.29 (-ve) 4.DQB1*03: No association. |
| Gregoire et al. (1994) | 66 (SCC) | 214 | African-American | PCR-SSO | PCR | 1. DQB1*03; RR=2.3 2. DQB1*0303 ; RR=5.2 3. DQB1*0604; RR=5.2 |

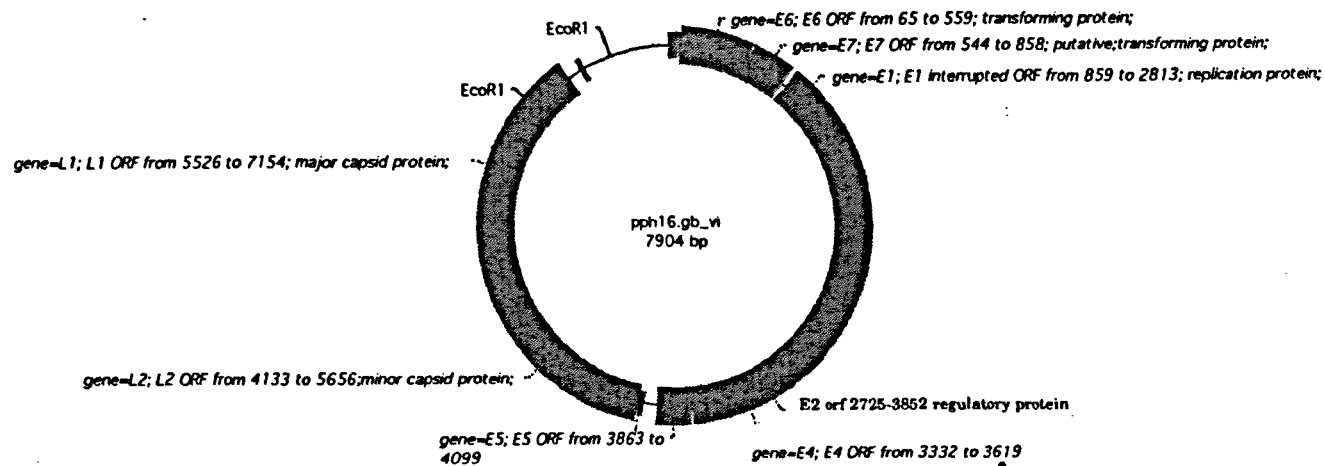


Fig 1.1: Schematic presentation of the HPV 16 genome. The numbers indicate the first and last nucleotide of the different open reading frames (ORF). E = early ORF, L = late ORF.

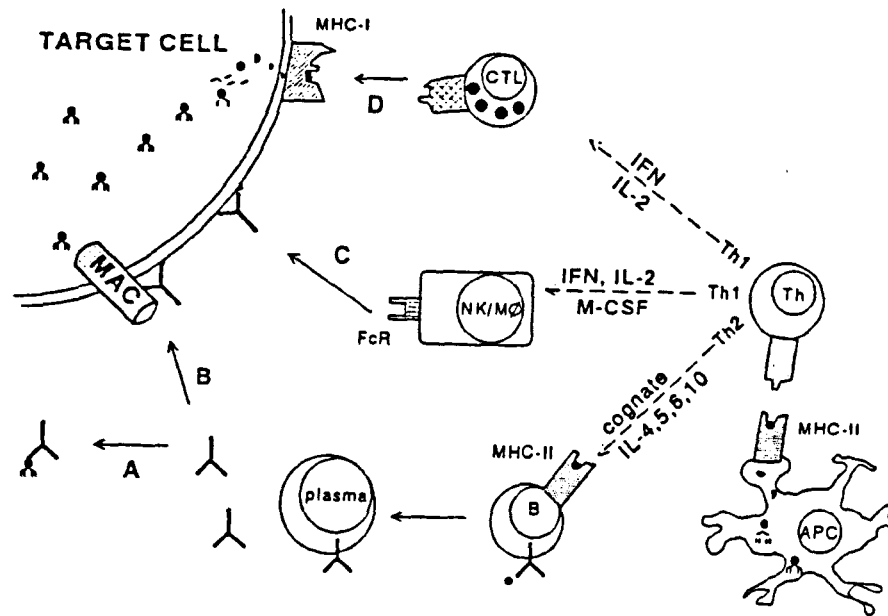


Fig 1.2: Antigen presentation by HLA class II molecules on professional APCs leads to T-helper activation. Subsequently different Th subsets stimulate different effector function: Th1 cells stimulate NK cells and macrophage activity by lymphokine production (g-IFN, IL-2 and M-CSF). In addition, Th1 stimulate clonal expansion of primed CTLs by IL-2 and g-IFN production. Th2 cells stimulate primed B-cells to proliferate and produce antibodies by the secretion of IL-4,5,6 and 10. Alternatively, the interaction between the T-cell receptor of Th2 and HLA class II on the activated B-cell leads to antibody production (cognate interaction pathway). Arrows indicated A-D represent different effector pathways: A, viral particle neutralization by antibodies; B, antibody-directed complement fixation, resulting in membrane damage by MAC; C, antibody dependent cell-mediated cytotoxicity (ADCC), in which NKs and macrophages bind via their Fc-receptor to antibodies adhered to the target cell; D, MHC-I restricted CTL mediated killing.

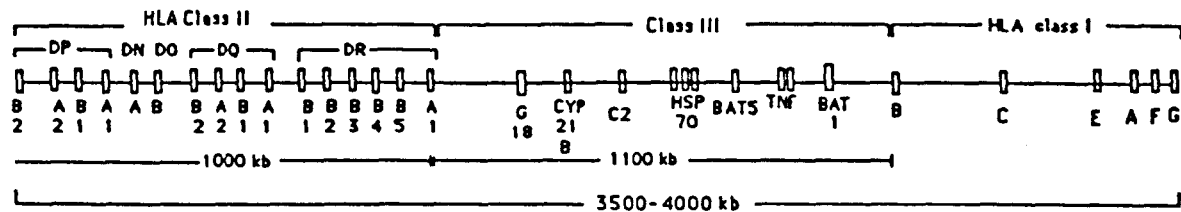


Fig 1.3: The HLA gene complex showing the 3500-4000kb of DNA with the locations and distances of Class II, Class I, complement (C2,C4, Bf, hydroxylase genes 21B and 21A), the heat shock protein genes 70 (HSP70), tumour necrosis factor, and HLA-B-associated transcripts (BATS).

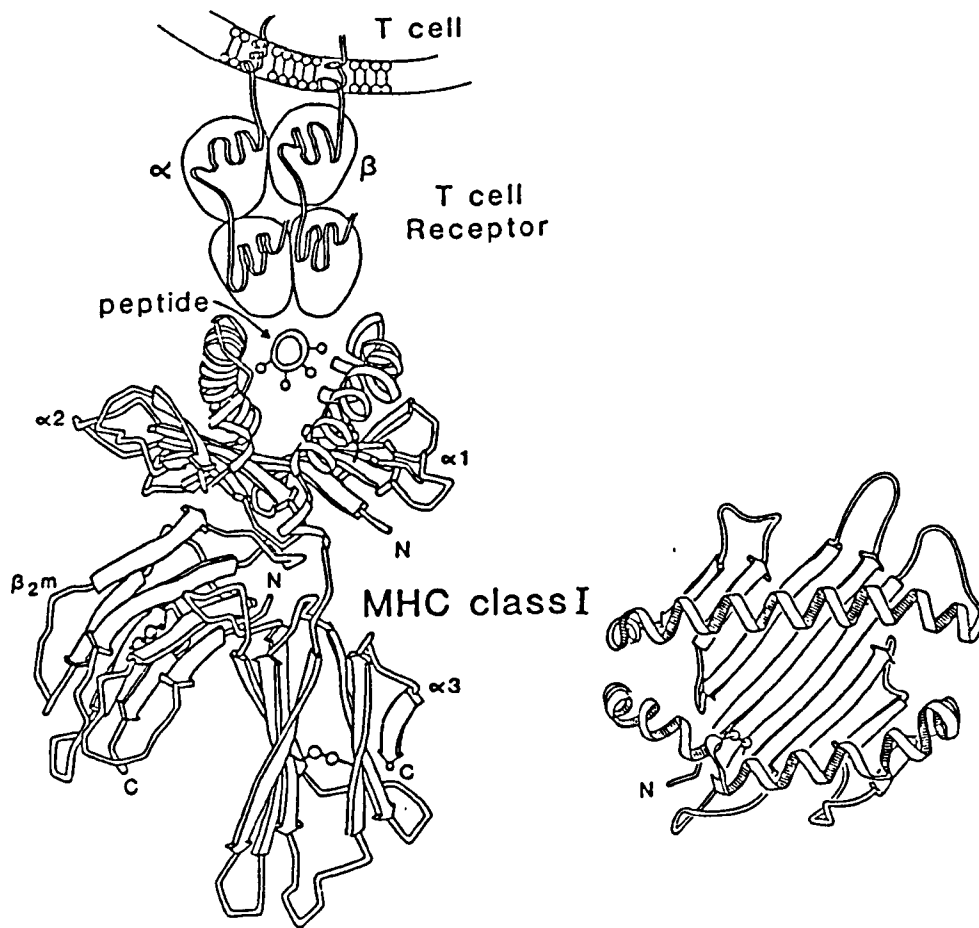


Fig 1.4: A representation of the trimolecular relationship between the MHC molecule, peptide, and T cell receptor. The class I molecule is shown. Class II molecules have a similar structure but different domain organization. On the right side, a top view of the peptide-binding site which consists of a β -pleated sheet formed by eight anti-parallel β strands, and the sides are formed by two α helical segments. Polymorphic residues in both Class I and Class II proteins are clustered in this peptide-binding region and are responsible for the different peptide specificities observed for different HLA proteins.

CHAPTER 2: METHODS AND MATERIALS

2.1 SAMPLE COLLECTION AND PREPARATION

2.1.1 PATIENT POPULATION

2.1.2 DNA PREPARATION

2.2 HPV TYPING

2.2.1 POLYMERASE CHAIN REACTION WITH TYPE SPECIFIC PRIMERS

2.2.2 PCR AMPLIFICATION CONDITIONS

2.2.3 PCR TEMPERATURE CONDITIONS

2.2.4 AGAROSE GEL ELECTROPHORESIS

2.3 HLA DQB1*03 TYPING : ARTIFICIAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (ARFLP)

2.3.1 PRINCIPLES OF ARFLP

2.3.2 DESIGN OF PRIMERS FOR ARFLP

2.3.3 PCR AMPLIFICATION CONDITIONS

2.3.4 RESTRICTION ANALYSIS

2.3.5 RESTRICTION ENZYME DIGESTION

2.4 POLYMERASE CHAIN REACTION WITH SEQUENCE SPECIFIC PRIMERS (PCR-SSP) FOR HLA DQB1*03 SUBTYPING

2.4.1 PRINCIPLES OF PCR-SSP

2.4.2 PRIMERS FOR AMPLIFICATION OF DQB1*03 ALLELES

2.4.3 PCR AMPLIFICATION CONDITIONS

2.5 POLYMERASE CHAIN REACTION-DIGOXIGENIN LABELED OLIGONUCLEOTIDE HYBRIDIZATION FOR HLA DQ-DR TYPING

2.5.1 INTRODUCTION AND PRINCIPLES

2.5.2 DIGOXIGENIN LABELING OF SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBES

- 2.5.3 HLA DQB GENERIC AMPLIFICATION
- 2.5.4 HLA DRB GENERIC AMPLIFICATION
- 2.5.5 PREPARATION OF DOT BLOTS
- 2.5.6 PREHYBRIDIZATION / HYBRIDIZATION AND TMACI WASHES
- 2.5.7 CHEMILUMINESCENT DETECTION OF DIGOXIGENIN LABELED PROBES WITH CSPD
- 2.5.8 STRIPPING OF MEMBRANES
- 2.5.9 HLA DRB GROUP SPECIFIC AMPLIFICATION
- 2.5.10 SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DQB TYPING
 - 2.5.11.1 HLA DQB PROBE SPECIFICITY
- 2.5.11 SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DRB
 - 2.5.11.1 HLA DR PROBE SPECIFICITY
- 2.6 HAPLOTYPIC ASSIGNMENT
- 2.7 STATISTICAL ANALYSIS
- 2.8 CELL LINES AND CULTURE CONDITIONS
 - 2.8.1 JHF CELL LINE
 - 2.8.2 JESTHOM CELL LINE
 - 2.8.3 CELL CULTURE CONDITIONS
- 2.9 IMMUNOAFFINITY PURIFICATION
 - 2.9.1 ANTIBODY FOR IMMUNOAFFINITY PURIFICATION
 - 2.9.2 TECHNIQUE OF AFFINITY CHROMATOGRAPHY
- 2.10 PEPTIDE ELUTION
- 2.11 SEPARATION OF PEPTIDES: REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (r-HPLC)
- 2.12 PEPTIDE SEQUENCING
 - 2.12.1 EDMAN DEGRADATION
- 2.13 SOLUTIONS AND REAGENTS

2.14 DNA CONTROL KITS

2.15 CHEMICAL AND MATERIAL SUPPLIERS

2.1 SAMPLE COLLECTION AND GENOMIC DNA EXTRACTION

2.1.1 SAMPLE COLLECTION

Cervical smears were taken from healthy women and those with CIN attending the outpatient clinics at City Hospital, Nottingham; Whittington Hospital, London, and the Margaret Pyke Center, London. In most cases, the referral for colposcopy was based on current British guidelines, i.e. a single moderate or severely dyskaryotic smear or a persistent mild abnormality. At the time of colposcopy, another smear was taken with an Ayre spatula and sent for routine cytological examination. The same spatula was used to collect additional cells, which were then agitated in phosphate buffered saline, and stored at -20°C. Any areas of abnormal epithelium found on colposcopy were biopsied (punch biopsy, loop diathermy, or laser cone as appropriate), and sent for routine histological assessment. Women with no visible colposcopic abnormality were not biopsied and were assumed to be histologically normal. Patients with colposcopic and histologic diagnosis of CIN formed the test population. Patients with normal cervical cytology who tested negative for HPV infection formed the control population. Histological classification into normal, CIN1, and CIN III were carried out according to established criteria^{6,277}.

2.1.2 GENOMIC DNA PREPARATION

After thawing, exfoliated cells were pelleted and washed twice in PBS. Cell pellets were digested with SDS (0.5%) and proteinase K (500µg/ml) for 6 hours or overnight at 37°C. An equal volume of equilibrated phenol was added and the solution was mixed with gentle rocking for 30 min at room temperature. The aqueous layer was removed by suction using a wide-bore pipette and re-extracted with phenol two to three times until the interface was clear. The aqueous layer was extracted once with an equal volume of phenol/chloroform and once more with chloroform. DNA was precipitated from the aqueous phase by the addition of two volumes of absolute alcohol, washed once with 70% ethanol and resuspended in 10mM Tris (pH 8) and 1mM EDTA (TE) and digested with 100µg/ml of RNase for 1 hour at 37°C. After re-extraction (once with phenol, once with phenol/chloroform and once with chloroform), the DNA was precipitated, washed with 70%

ethanol and dissolved in 50µl of TE. The amount of DNA recovered from each specimen was determined by spotting 1µl of serial dilutions on a commercially available dipstick (Invitrogen).

2.2 HPV TYPING

2.2.1 POLYMERASE CHAIN REACTION WITH TYPE SPECIFIC PRIMERS

Separate PCR reaction were run for each of the HPV types 16, 18, 31, 33 using the primers shown in table 2.1. The PCR primers were chosen from the literature to be type specific. This was confirmed using cloned HPV plasmids and by the results obtained on some clinical specimens using alternative type specific primer pairs which gave entirely consistent results.

2.2.1.1 PCR AMPLIFICATION CONDITIONS

PCR amplifications were performed in either a Techne PHC-3 or Perkin Elmer Cetus machine. The reactions were performed in 50µl containing 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, 0.01% gelatin and 50pmol of each primer. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. 1.25 units of Ampli Taq polymerase (Perkin Elmer Cetus) was added at 70°C after the initial denaturation.

2.1.1.2 PCR TEMPERATURE CONDITIONS

HPV 16: Sense and antisense primers for HPV 16 were used with the conditions described by Seedorf et al²⁷⁸. Initial denaturation was for 8 minutes followed by 35 cycles of 94°C for 15 seconds, 54°C for 15 seconds and 72°C for 30 seconds, with a final extension at 72°C for 8 minutes.

HPV 18: The primers for HPV 18 were used with the conditions described by Coles and Danos²⁷⁹. Initial denaturation was for 8 minutes followed by 35 cycles of 94°C for 15 seconds, 70°C for 15 seconds and 72°C for 30 seconds, with a final extension at 72°C for 8 minutes.

HPV 31: Sense and antisense primers for HPV 31 were used with the conditions described by Goldsborough et al²⁸⁰. Initial denaturation was for 8 minutes followed by 35 cycles of 94°C for 15 seconds, 54°C for 15 seconds and 72°C for 30 seconds, with a final extension at 72°C for 8 minutes.

HPV 33: Sense and antisense primers for HPV 33 were used with the conditions described by Cole and Streeck²⁸¹. Initial denaturation was for 8 minutes followed by 35 cycles of 94°C for 15 seconds, 65°C for 15 seconds and 72°C for 30 seconds, with a final extension at 72°C for 8 minutes.

2.2.1.3 AGAROSE GEL ELECTROPHORESIS

Agarose (Sigma) gels of 2% concentration (wt/vol) were made with and run in 1X Tris-acetate-EDTA (TAE) buffer pH 8.0²⁸². The PCR product were mixed with 2µl of loading dye²⁸² and loaded into the wells of the gel. The gels were run at room temperature at a constant voltage of 70v for approximately three hours. Variations of both voltage and run times were used for convenience and for better resolution of the DNA fragments. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm. The gels were photographed. As an aid to fragment size identification, 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included.

2.3 HLA DQB1*03 TYPING: ARTIFICIAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (A-RFLP)

2.3.1 PRINCIPLES OF A-RFLP

Restriction analysis of PCR products is one of the earliest techniques used for analyzing amplified products²⁰⁹. This approach is applicable for distinguishing alleles in which the polymorphic residue results in the creation or removal of a restriction enzyme site. Unfortunately, many

polymorphisms are not associated with restriction enzyme site change and thus are not amenable to this analysis. However, by using site directed mutagenesis using primers with mismatches near the 3' ends, it is possible to create an artificial RFLP (A-RFLP) for almost all naturally occurring DNA polymorphisms²⁸³. Fig 2.1 illustrates the principles of this approach.

2.3.2 DESIGN OF PRIMERS FOR A-RFLP

An A-RFLP primer can be designed using a semi-automatic approach by using a computer programme which will search for restriction enzyme sites for a given sequence, e.g DNA Strider. The process is illustrated in Fig 2.2. If it is assumed that the polymorphic residue is P and restriction enzymes with recognition sites of up to 6 bases are needed. The five bases on either side of P are entered into the computer programme from -5 to +5 and the programme is used to search for a restriction enzyme site encompassing P. If a restriction enzyme site is found which is only present in one allele but not in the other one, then no further searching is required. If no restriction site polymorphism is found, then the nucleotides from -2 to -5 and +2 to +5 are changed one at a time with a computer search being carried out after each alteration. For each position, the nucleotide A, T, C and G is substituted in turn. The -1 or +1 position is avoided as this may reduce amplification efficiency and is used as the last base of the PCR primer. All possibilities are investigated as more than one solution may be possible for a given polymorphism and some restriction enzymes work better than others.

For HLA DQB1*03 primer design, all DQB1*03 alleles possess an A at the last base of codon 38 followed by CGC (Codon 39) and TTC (codon 40). Thus if the first base of codon 40 can be mutated from "T" to "G", then a *Mlu* I site (ACGCGT) will be created for the DQB1*03 alleles. The non DQB1*03 alleles, on the other hand, possess a "G" in the last base of codon 38. No *Mlu* I site will therefore be created by mutating the first base of codon 40 (Fig. 2.3). Following endonuclease restriction, the PCR product from the allele with the restriction site will have the portion containing the ARFLP primer cleaved off, thus resulting in smaller size fragment on gel

electrophoresis. The forward primer "A" is used in conjunction with the reverse mutagenesis primer "B".

A: 5' AGG GAT CCC CGC AGA GGA TTT CGT GTACC 3' (forward)

B: 5' CCG GTA CAC CCC CAC GTC GCT GTC GAC GCG 3' (reverse)

(The mutating base is underlined)

2.3.3 PCR AMPLIFICATION CONDITIONS

PCR amplifications were performed in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. The initial denaturation was at 94°C for 8 minutes followed by 30 cycles each at 94°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute. There was a final extension step at 72°C for 15 minutes. All PCR reactions were performed with both negative and positive controls.

2.3.4 RESTRICTION ANALYSIS

Following amplification 10µl of the PCR product was restricted with 50 units of *Mlu* I (Boehringer Mannheim) in a volume of 20µl at 37°C overnight using manufacturer's buffer. The products were analyzed by electrophoresis on 4% agarose gels (Metaphor, Flowgen). The agarose gels were made with and run in 1X TAE buffer, pH 8.0. The genomic digests were mixed with 2µl of loading dye and loaded into wells of the gel. For fragment size identification, 123 bp marker from *Eco*RI/*Bam* HI fragments of adenovirus type 2 was included. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm and the gels photographed.

2.4 POLYMERASE CHAIN REACTION WITH SEQUENCE SPECIFIC PRIMERS (PCR-SSP) FOR HLA DQB1*03 SUBTYPING

2.4.1 PRINCIPLES OF PCR-SSP

PCR amplification of the HLA DQ locus with sequence specific primers is a powerful method for detecting genetic variability, including single base pair mismatches. The technique is based on the principle that a completely matched primer will be more efficiently utilized in the PCR reaction than a primer with one or several mismatches in the 3' end. The resolution of the method is high, especially in heterozygotes, as each primer pair identifies two sequence motifs located on the same chromosome, i.e. in *cis*. The post amplification processing of samples consists of determining whether amplification has occurred or not, since the discrimination between alleles takes place during the enzymatic *in vitro* DNA amplification. The PCR-SSP technique for HLA DQ typing was introduced by Olerup et al²⁸⁴ with good reproducibility, and the results were 100% concordant with allelic assignment by *Taq* I DRB-DQA-DQB haplotype analysis.

2.4.2 PRIMERS FOR AMPLIFICATION OF DQB1*03 ALLELES

Eight primer pairs (Table 2.2) were used to identify the DQB1*03 alleles. The primers were defined by Olerup et al²⁸⁴ based on the nucleotide sequences of the first 92 amino acids of the DQB1 alleles .

2.4.3 PCR AMPLIFICATION CONDITIONS

PCR amplifications were performed in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, and 1U of *Taq* DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. The initial denaturation was at 94°C for 8 minutes followed by 30 cycles each at 94°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute. There was a final extension step at 72°C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology

(BISHI). Agarose gel electrophoresis was performed as described above. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm. The gels were photographed. 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included.

2.5 POLYMERASE CHAIN REACTION - DIGOXIGENIN LABELED OLIGONUCLEOTIDE HYBRIDIZATION FOR HLA DQ-DR TYPING

2.5.1 INTRODUCTION AND PRINCIPLES

The amplification of specific DNA sequences by polymerase chain reaction followed by hybridization with sequence-specific oligonucleotide probes (SSO) has become a powerful technique for detailed analysis of genetic variations^{209,285}. Each probe is constructed so as to be exactly complementary to an allele specific motif within one of the hypervariable regions of the exon. When hybridized under the appropriate conditions, these synthetic DNA probes (usually 15-20 bases in length) will anneal to their complementary target sequences in the sample DNA only if they are completely matched. The PCR product is denatured, spotted onto a charged nylon membrane, hybridized with a labeled SSO probe, washed at a stringent temperature and examined by an autoradiographic, colourimetric or chemiluminescence assay. In the case of an absolute nucleotide sequence match between the SSO probe and the membrane bound target DNA, washing at a stringent temperature fails to denature the probe-target hybrid and this is shown by a positive signal from the probe. A mismatch of one or more nucleotides results in denaturation of the probe-target hybrid and elution of the labeled probe, and consequently, no signal is generated. With an appropriate selection of oligonucleotide probes, the relevant genetic content of a DNA sample can be completely described.

The temperature and salt concentration at which the membrane is washed are influenced by the nucleotide composition and length of individual SSO probes, and therefore a variety of probe specific washing temperatures are used. The allele specificity of the target DNA may thus be determined using a series of SSO probes. This requires the preparation of replicate membranes, one for each probe to be tested. Alternatively, a single or small number of membranes may be used, necessitating the removal of each probe after signal development, before reprobing with another SSO.

Traditionally, the PCR-SSO technique has relied on 5'- end labeling (usually ^{32}P -labeled) of the SSO. Radioactive labeling is associated with several disadvantages and a number of non-radioactive alternatives have become available. In these systems (see section 1.4.1), probe target hybridization is revealed by the use of reporter molecules such as streptavidin-enzyme or specific antibody-enzyme conjugates, in colourimetric or chemiluminescence assays. The complexity of the dot-blot PCR-SSO typing system is proportional to the number of SSO probes required to discriminate between each allele at a given locus.

The 11th Histocompatibility Protocol for PCR-SSO²¹⁶ with some modifications have been used in this work. A complete listing of probes and reagents are at the end of this chapter.

2.5.2 DIGOXIGENIN LABELING OF SSO PROBES

Digoxigenin is a steroid hapten (Fig 2.4). DNA probes may be labeled with DIG-11-dUTP via random primed labeling, nick translation, cDNA synthesis or Taq DNA polymerase. Oligonucleotide probes can be 3'-end labeled with DIG-11-ddUTP, tailed with DIG-11-dUTP by terminal transferase. In this study, probes were labeled at the 5' end with Digoxigenin-NHS Ester (Digoxigenin-3-O-methylcarbonyl-E aminocaproic acid-N-hydroxysuccinimide Ester).

The synthesis and labeling of these probes was done by Dr. Ian Goldsmith at the Clare Hall Laboratories of the Imperial Cancer Research Fund. The oligonucleotide was synthesized and deprotected according to standard protocol by treatment with 25% aqueous ammonia which was subsequently removed by lyophilization. Ethanol precipitation was performed by dissolving the oligomer in a mixture of 300µl of distilled water and 30µl of sodium acetate buffer, 3mol/l; pH 8.5, and transferred to a microfuge tube. 9ml of ice cold ethanol was added, mixed and kept at -20°C for 2 hours. The solution was centrifuged for 15 minutes at 10,000g and the supernatant decanted. The pellet was washed with 100µl of ice-cold ethanol, centrifuged for 5 min and the supernatant was removed. The pellet was dissolved in 200µl of sodium borate buffer, 0.1mol/l; pH 8.5. 1mg of Digoxigenin-NHS Ester was dissolved in 600µl of ethanol, and 200µl of this solution was added to the solution of oligonucleotide and kept overnight at ambient temperature in a shaker. Separation of labeled oligonucleotide from the unlabeled compound was achieved by using reversed phase HPLC.

2.5.3 HLA DQB GENERIC AMPLIFICATION

Generic HLA DQB1 amplification was performed using primers:

DQBAMP-A 5'CATGTGCTACTTCACCAACGG-3' and

DQBAMP-B 5'CTGGTAGTTGTGTCTGCACAC-3'

PCR amplifications were performed in 96 well microtitre plates in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 2.0mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. Each final reaction mixture was overlaid with several drops (about 30µl) of mineral oil. The initial denaturation was at 95°C for 5 minutes followed by 35 cycles each at 95°C for 45 seconds, 60°C for 1 minute and 72°C for 1 minute. There was a final extension step at 72°C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology (BISHI).

2.5.4 HLA DRB GENERIC AMPLIFICATION

Generic HLA DRB amplification was performed using primers:

DRBAMP-A 5'CCCCACAGCACGTTTCTTG-3' and

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

PCR amplifications were performed in 96 well microtitre plates in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 2.0mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. Each final reaction mixture was overlaid with several drops (about 30µl) of mineral oil. The initial denaturation was at 95°C for 5 minutes followed by 35 cycles each at 95°C for 45 seconds, 60°C for 1 minute and 72°C for 1 minute. There was a final extension step at 72°C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology (BISHI).

After completion of thermal cycles, an aliquot (3µl) of each reaction sample was subject to agarose gel electrophoresis. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm. The gels were photographed. 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included. Generic DQB1 amplification generated a 214bp fragment while DRB1 generated a 274bp fragment.

2.5.5 PREPARATION OF DOT BLOTS

Hybond-N positively charged nylon membranes (Amersham International plc, Aylesbury, Bucks, UK) were used. The membranes were cut to appropriate size allowing 1cm² per dot, corresponding to the size of a 96 well microtitre plate. The membranes were not prewetted.

PCR products were heated to 95 degrees for 10 minutes and placed on ice. Spotting was performed using the Biomek 1000 Laboratory Automation Workstation. The equipment was programmed to perform multi-tip pipetting of 2 μ l PCR products from microtitre plates and dotted on the nylon membranes. Using its 8 channel pipetting tool, the Biomek spots samples on to the membrane held on a purpose made vacuum blotter. Spots are placed in an 8x12 array for compatibility with a standard 96-well microtitre plate. The membranes were allowed to air dry for at least 10 minutes before being placed in a UV cross linker. DNA cross linking was performed using the auto power setting. This provides 254nm UV lamp of 0.12J/cm². The membranes are stored at 4⁰C until required.

2.5.6 PREHYBRIDIZATION/HYBRIDIZATION AND TMACI WASHES

The baked membranes were placed in 50ml Falcon tube with no overlap. The membrane was blocked in 5ml blocking solution (Boehringer Mannheim) at room temperature on rotisserie for at least 30 mins. The blocking solution was poured off and to the tube was added 5ml prehybridization solution {4X SSPE, 0.1% laurylsarcosine, 1% blocking reagent}, 50 μ l (10mg/ml) sonicated/boiled salmon sperm DNA, which has been preheated to appropriate temperature (52⁰C for DQB and 54⁰C for DRB). Prehybridization was performed for 1 hour. The solution was poured off and 2pM SSO (listed below) per ml of hybridization solution was added to the tube and incubated at appropriate temperature for 1 hour 30 minutes (52⁰C for DQB and 54⁰C for DRB).

The hybridized membranes were removed from the tubes and washed twice in 1L 2X SSPE/0.1% SDS for 10 minutes at room temperature, in trays on an orbital shaker. The membranes were then washed twice in 50mM Tris (pH 8), 0.1% SDS, 2mM EDTA (pH8), 3M TMACI (Tetramethylammonium chloride, Sigma) solution at 58⁰C. This allows A-T rich probe to remain annealed at 10⁰C higher than the predicted temperature of dissociation. Also, as a means of standardizing posthybridization washing temperatures, TMACI was used in the washing solutions.

It allows a common washing temperature for each probe used, provided that they contain the same number of nucleotides. The membranes were gently blotted and stored moist in polythene at 5°C. Once washed, the membranes were stored for up to 24 hours before the detection procedure.

2.5.7 CHEMILUMINESCENT DETECTION OF DIGOXIGENIN LABELED PROBES WITH CSPD

Disodium 3-(4-methoxy-3-(2-(5-chloro-2-tricyclo

[3.3.1.13,7]decan-4-yl) phenyl phosphate (CSPD, Boehringer Mannheim), is a chemiluminescent substrate for alkaline phosphatase that enables sensitive and fast detection of biomolecules by producing visible light which is recorded on film. Enzymatic dephosphorylation of CSPD by alkaline phosphatase leads to the metastable phenolate anion which decomposes and emits light at a wavelength of 477nm.

All steps were carried out at room temperature. The membranes were washed in 1L of buffer 1 in a tray on an orbital shaker for at least 5 minutes. The membranes were blotted dry and placed in clean plastic tubes dot side up. 5 ml of buffer 2 was added to each tube and placed on rotisserie for at least 30 minutes. 1µl of Anti-digoxigenin-Alkaline Phosphatase, Fab fragments (Boehringer Mannheim) was added to the solution (1:10,000 dilution). The tubes were placed on rotisserie for 40 minutes. The membranes were then washed thrice in buffer 1 to remove any excess Anti-DIG fragments. The membranes were blotted dry, placed in plastic tubes and equilibrated in buffer 3 for 5 minutes. For membranes that required to be reprobbed, buffer 3 was used without magnesium.

CSPD was prepared by diluting the 10mg/ml solution in buffer 3, 1:100 and placed in a container with a large surface area to volume ratio. The membranes were placed face down in solution, for 5 minutes, ensuring there were no air bubbles at the interface. The membranes were removed and gently blotted dry. For the briefest exposure to X-ray film, the alkaline phosphatase chemiluminescent reaction must be at a steady state. This was brought about by a 15 minute

incubation at +37⁰C. DRB probes were exposed at 45 minutes and DQB at 1 hour 30 minutes. Because not all of the SSO may be labeled to the same extent, long exposure (12 hours) was also performed.

2.5.8 STRIPPING OF MEMBRANES

Membranes were stripped to enable reprobing. The membranes were incubated twice for 10 minutes in 0.2N NaOH, 0.1% SDS solution at 37⁰C. This incubation removed the DIG-labeled probe. The membranes were then rinsed thoroughly in 2X SSPE for 15minutes. They were either stored moist at 4⁰C or reprobing was commenced with the prehybridization step of the desired hybridization procedure.

2.5.9 HLA DRB GROUP SPECIFIC AMPLIFICATION

From the hybridization patterns in response to the SSO, individuals were assigned as belonging to one or more of the following groups.

- A. DR1 group
- B. DR2 group
- C. DR4 group
- D. DR52 associated group (DR3, DR5, DR6, DR8).
- E. DR 52 group

The DRB1 genes that can be typed directly by the generic amplification procedure are DRB1*07 (corresponding to DRB1*0701 or DRB1*0702), DRB1*0901, and DRB1*1001. The DRB3*0101 and DRB4*0101 can also be assigned for the DRB3 and DRB4 genes respectively. Further subtyping utilizes group specific amplification followed by SSO hybridization.

For group specific amplification, samples from the different groups were amplified as follows.

- A. DR1 group with DR1-DRB1 specific primer pair

DRBAMP-1 5'TTCTTGTGGCAGCTTAAGTT-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

B. DR2 group with DR2-DRB1 specific primer pair

DRBAMP-2 5'TTCCTGTGGCAGCCTAAGAGG-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

C. DR4 group with DR4-DRB1 specific primer pair

DRBAMP-4 5'GTTTCTTGGAGCAGGTAAAC-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

D. DR52-associated group with DR52-associated group-DRB1 specific primer

DRBAMP-3 5' CACGTTTCTTGGAGTACTCTAC-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

E. DR52 group with DR52-DRB3 specific primer

DRBAMP-52 5' CCCAGCACGTTTCTTGGAGCT

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

PCR amplifications were performed in 96 well microtitre plates in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 2.0mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. Each final reaction mixture was overlaid with several drops (about 30µl) of mineral oil. The initial denaturation was at 95⁰C for 5 minutes followed by 35 cycles each at 95⁰C for 45 seconds, 60⁰C for 1 minute and 72⁰C for 1 minute. There was a final extension step at 72⁰C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology (BISHI).

Each reaction sample was subject to agarose gel electrophoresis. The product sizes were 261, 261, 263, 266 and 271 for DR1-DRB1, DR2-DRB1, DR4-DRB1, DR52 associated-DRB1, and DR52-

DRB3 respectively. Oligonucleotide hybridization of the amplified products were performed as described above using the SSO probes in section 2.5.12.

2.5.10 SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DQB TYPING

| | |
|----------|-------------------------|
| DQB2301 | GAC CGA GCT CGT GCG GGG |
| DQB2302N | AAC GGG ACC GAG CGC GTG |
| DQB2601 | CGG GGT GTG ACC AGA CAC |
| DQB2602 | CGT TAT GTG ACC AGA TAC |
| DQB2603 | CGT CTT GTG ACC AGA TAC |
| DQB2604 | CGT CTT GTA ACC AGA CAC |
| DQB2605 | CGT CTT GTG AGC AGA AGC |
| DQB2606 | CGT CTT GTA ACC AGA TAC |
| DQB3701 | AGG AGT ACG TGC GCT TCG |
| DQB3702N | AGG AGG ACG TGC GCT TCG |
| DQB3703 | TAA CCG AGA AGA GTA CGT |
| DQB4501N | GAC GTG GAG GTG TAC CGG |
| DQB4901 | GGT GTA CCG GGC AGT GAC |
| DQB4902N | GGT GTA TCG GGC GGT GAC |
| DQB5701 | GCG GCC TGT TGC CGA GTA |
| DQB5702 | GCG GCC TAG CGC CGA GTA |
| DQB5703 | GGC GGC CTG ACG CCG AGT |
| DQB5704 | GCG GCC TGA TGC CGA GTA |
| DQB5705 | GGC TGC CTG CCG CCG AGT |
| DQB5706 | GGC CGC CTG ACG CCG AGT |
| DQB5707 | GGC CGC CTG CCG CCG AGT |
| DQB5708 | GCG GCT TGA CGC CGA GTA |
| DQB7001 | GAC CCG AGC GGA GTT GGA |

DQB7003 GAG GGG ACC CGG GCG GAG

DQB7005 GAA ACG GGC GGC GGT GGA

2.5.10.1 HLA DQB PROBE SPECIFICITY

| Probe Name | HLADQ Specificity |
|-------------|--------------------------|
| 1. DQB2301 | 0401 |
| 2. DQB2302 | 03031,0402 |
| 3. DQB2601 | 0501, 0502, 05031, 05032 |
| 4. DQB2604 | 0603, 0604 |
| 5. DQB2606 | 0605 |
| 6. DQB3702N | 0601 |
| 7. DQB4901 | 0501 |
| 8. DQB5701 | 0501, 0604, 0605 |
| 9. DQB15702 | 0502, 0504 |
| 10. DQB5703 | 05031, 0601 |
| 11. DQB5704 | 05032, 0602, 0603 |
| 12. DQB5705 | 0201 |

2.5.11 SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DRB

DRB1005 AGA AAT AAC ACT ACA CCG

DRB1006 TGG CAG GGT AAG TAT AAG

DRB1007 GAA GCA GGA TAA GTT TGA

DRB1008 GAG GAG GTT AAG TTT GAG

DRB2802 GGT TAC TGG AGA GAC ACT

DRB2807 GCG GTA CCT GGA CAG ATA

DRB 2810 GCG AGT GTG GAA CCT GAT

| | |
|---------|-------------------------|
| DRB3701 | CCA AGA GGA GTC CGT GCG |
| DRB3707 | AAC CAA GAG GAG AAC GTG |
| DRB3712 | CAG GAG GAG TTC GTG CGC |
| DRB3713 | GCG CAC GTA CTC CTC TTG |
| DRB5701 | GCC TGA TGC CGA GTA CTG |
| DRB5702 | GCC TAG CGC CGA GTA CTG |
| DRB5703 | GCC TGA TGA GGA GTA CTG |
| DRB5704 | GCC TGC TGC GGA GCA CTG |
| DRB5705 | GCC TGT CGC CGA GTC CTG |
| DRB5708 | GCC TGA TGC TGA GTA CTG |
| DRB7001 | TCC TGG AGC AGA GGC GGG |
| DRB7002 | GAC TTC CTG GAA GAC AGG |
| DRB7003 | GAC CTC CTG GAA GAC AGG |
| DRB7004 | GGC CGG GTG GAC AAC TAC |
| DRB7005 | ACC GCG GCC CGC TTC TGC |
| DRB7006 | GCA GAG GCG GGC CGA GGT |
| DRB7007 | ACA TCC TGG AAG ACG AGC |
| DRB7008 | ACT TCC TGG AAG ACG AGC |
| DRB7009 | AGC GGA GGC GGG CCG AGG |
| DRB7011 | GAC ATC CTG GAG CAG GCG |
| DRB8601 | AAC TAC GGG GTT GGT GAG |
| DRB8602 | AAC TAC GGG GCT GTG GAG |
| DRB8603 | AAC TAC GGG GTT GTG GAG |

2.5.11.1 HLA DRB PROBE SPECIFICITY

A. Generic

| Probe name | HLA DR specificity |
|------------|--------------------|
| 1. DRB1001 | DR1 |
| 2. DRB1009 | DRB5 |

| | |
|--------------|-----------------------------------|
| 3. DRB1004 | DR4 |
| 4. DRB1010N | DRB3*0101 |
| 5. DRB1006 | DR7 |
| 6. DRB1007 | DR9 |
| 7. DRB1008 | DR10 |
| 8. DRB2802 | DR12 |
| 9. DRB3709 | DRB5*0101 |
| 10. DRB5703 | DR11 |
| 11. DRB2810 | DR53 |
| 12. DRB1003 | DR52 Associated group (DR3, DR11, |
| DR13,DR14) | |
| 13. DRB1005 | DR52 Associated group (DR12, DR8, |
| DR14) | |
| 14. DRB 1011 | DRB3*0201+ DRB3*0202 |
| 15. DRB1002 | DRB3*0301 |

B. GROUP SPECIFIC

(i) HLA DRB1*04 PROBES

| | Probe 3701 | Probe 5701 | Probe 5702 | Probe 7001 | Probe 7005 | Probe 7006 | Probe 7007 | Probe 8601 |
|-----------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| DRB1*0401 | - | + | - | - | + | - | - | + |
| DRB1*0402 | - | + | - | - | - | - | + | - |
| DRB1*0403 | - | + | - | + | - | + | - | - |
| DRB1*0404 | - | + | - | + | - | - | - | - |
| DRB1*0405 | - | - | + | + | - | - | - | + |
| DRB1*0406 | + | + | - | + | - | + | - | - |
| DRB1*0407 | - | + | - | + | - | + | - | + |
| DRB1*0408 | - | + | - | + | - | - | - | + |
| DRB1*0409 | - | - | + | - | + | - | - | + |
| DRB1*0410 | - | - | + | + | - | - | - | - |
| DRB1*0411 | - | - | + | + | - | + | - | - |

(ii) HLA DR2/DRB5 PROBES

| | Probe 2813 | Probe 3707 | Probe 7002 | Probe 7003 | Probe 8601 | Probe 8603 |
|-----------|---------------|---------------|---------------|---------------|---------------|---------------|
| DRB1*1501 | + | - | - | - | - | + |
| DRB1*1502 | + | - | - | - | + | - |
| DRB1*1601 | + | - | + | - | + | - |
| DRB1*1602 | + | - | - | + | + | - |
| DRB1*1503 | - | - | - | - | - | + |
| DRB5*0101 | - | - | - | - | + | - |
| DRB5*0102 | - | + | - | - | + | - |
| DRB5*0201 | - | + | - | - | - | - |
| DRB5*0202 | - | + | - | - | - | - |

(iii) HLA DR1 PROBES

| | Probe 7001 | Probe 7007 | Probe 8602 |
|-----------|------------|------------|------------|
| DRB1*0101 | + | - | - |
| DRB1*0102 | + | - | + |
| DRB1*0103 | - | + | - |

(iv) DRB1-ASSOCIATED DRB1 PROBES

| | Probe 1003 | 1005 | 1013 | 2802 | 2807 | 2809 | 2813 | 3707 | 3712 | 3713 | 5701 | 5702 | 5703 | 5704 | 5705 | 5708 | 7001 | 7002 | 7003 | 7004 | 7007 | 7008 | 7009 | 7010 | 8601 | 8602 | 8603 |
|------------|------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| DRB1*0301 | + | - | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | + |
| DRB1*0302 | + | - | - | - | - | + | - | + | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - | + | - | - |
| DRB1*1101 | + | - | - | - | - | - | + | - | - | + | - | - | + | - | - | - | - | + | - | - | - | - | - | - | + | - | - |
| DRB1*1102 | + | - | - | - | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - | + | - | - | c | - | - | + | - |
| DRB1*1103 | + | - | - | - | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | + | - |
| DRB1*1104 | + | - | - | - | - | - | + | - | - | + | - | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - | + |
| DRB1*1201 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | + | - | + | - |
| DRB1*1202 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - | - | - | + | - |
| DRB1*1301 | + | - | - | - | - | - | + | + | - | - | + | - | - | - | - | - | - | - | - | + | - | - | c | - | - | + | - |
| DRB1*1302 | + | - | - | - | - | - | + | + | - | - | + | - | - | - | - | - | - | - | - | + | - | - | c | + | - | - | - |
| DRB1*1303 | + | - | - | - | - | - | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | c | + | - | - | - |
| DRB1*1304 | + | - | - | - | - | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | c | - | - | + | - |
| DRB1*1305 | + | - | - | - | - | - | + | + | - | - | + | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - |
| DRB1*1401 | + | - | - | - | - | - | + | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | + |
| DRB1*1402 | + | - | - | - | - | + | - | + | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - |
| DRB1*1403 | + | - | - | - | - | + | - | + | - | - | + | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - |
| DRB1*1404 | - | + | - | - | - | - | + | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | + |
| DRB1*1405 | + | - | + | - | - | - | + | - | + | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - | - | + |
| DRB1*1406 | + | - | - | - | - | + | - | + | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + |
| DRB1*1407 | + | - | - | - | - | - | + | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - | + | - | + | - | - |
| DRB1*1408 | + | - | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | + |
| DRB1*0801 | - | + | - | - | - | - | + | - | - | + | - | + | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - |
| DRB1*0802 | - | + | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - |
| DRB1*0803 | - | + | - | - | - | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| DRB1*08031 | - | + | - | - | - | - | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| DRB1*08042 | - | + | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | + |

c = Cross hybridized.

2.6 HAPLOTYPIC ASSIGNMENT

HLA DRB1, DRB3, DRB4, DRB5 and DQB1 haplotypes were inferred based on known patterns of linkage disequilibrium in Caucasians for these loci²⁸⁶⁻²⁸⁷. If a sample contained DRB1*0401, DRB1*0101 at the DRB1 locus and DQB1*0301, DQB1*0501 at the DQB1 locus, then the correct inferred haplotypes would be DRB1*0401-DQB1*0301 and DRB1*0101-DQB1*0501 which is known to occur naturally. For supertypic antigens HLA DRB3, DRB4 and DRB5, the 3-locus haplotypes were inferred²⁸⁷.

2.7 STATISTICAL ANALYSIS

Odds ratios and their approximate 95% confidence intervals were calculated for all variables by the χ^2 test for 2 x 2 tables without a continuity correction²⁸⁸. For small samples, exact 'p' values were calculated. For 2 x k tables, the χ^2 test for trend was calculated²⁸⁹. The unit of sampling was the allele in all analysis except when studying the effect of homozygosity versus heterozygosity. For other analysis, each allele or haplotype was taken as an independent observation so that the sample size was twice as large for these comparisons. No formal adjustments of 'p' values for multiple comparisons were made.

2.8 CELL LINES AND CULTURE CONDITIONS

In the present study, the haplotype HLADRB1*0401-DQB1*0301 was shown to correlate with susceptibility to HPV and CIN while DRB1*0101-DQB1*0501 indicated protection (Details of results in chapters 3 and 4). Although the binding motifs of several HLA-DR molecules have been defined, studies on the binding motifs of HLA-DQ molecules are few^{269,290-297}. Therefore, the peptide pools eluted from HLA {DQA1*0301/DQB1*0301} and {DQA1*0101/DQB1*0501} were sequenced. Amino acid preferences based on peptide sequence alignment with HPV 16 and polymorphic residue substitutions in the binding cleft of HLA DQ are discussed in chapters 5 and 6.

2.8.1 JHF CELL LINE

The JHF cell line, Xth International Histocompatibility Workshop No. 9030, is a B lymphoblastoid cell line obtained from the ECACC. The cell line is homozygous for the following HLA alleles:

HLA-A*31011; HLA-C*15; HLA-DRB1*0407; HLA-DRB4*0101; HLA-DQA1*0301; HLA-DQB1*0301; HLA-DPA1*01; HLA-DPB1*0301.

2.8.2 JESTHOM CELL LINE

The Jesthom cell line, Xth International Histocompatibility Workshop No. 9004, is a B lymphoblastoid cell line from the ECACC. The cell line is homozygous for the following HLA alleles:

HLA-C*01; HLA-DRA*0101; HLA-DRB1*0101; HLA-DRB6*0101; HLA-DQA1*0101; HLA-DQB1*0501; HLA-DPA1*01; HLA-DPB1*0401.

2.8.3 CELL CULTURE CONDITIONS

The cells were grown in RPMI 1640 supplemented with 5% fetal calf serum, 5% CO₂, 2% bicarbonate, 2mM glutamine, 50U/ml penicillin G, and 50µg/ml streptomycin in roller bottles at 37°C. Cultures were split every 3-7 days to two- to fivefold volume, depending on the expansion rate. When the required number of cells was reached (10¹¹), they were spun down at 1,000 r.p.m. The supernatant was removed and the pellet washed with PBSA. The wash was repeated twice and the final pellet was frozen at -80 degrees until used.

2.9 IMMUNOAFFINITY PURIFICATION

Immunoaffinity purification is a powerful technique for the isolation of proteins. Under proper conditions, purifications of 1,000 to 10,000-fold can be achieved in a single step. The factors that affect the success of the technique include the starting purity of the antigen,

the affinity of the antibody for the antigen, and the ease with which the antigen-antibody bond can be broken. The affinity of the antibody for the antigen determines the total amount of antigen that can be removed. For example, antibodies of high affinity ($>10^8$ / mol), quantitative removal can be achieved in less than 1 hour. Even at high antibody concentrations, low-affinity antibodies (10^6 /mol) will not bind all of the antigen in solution. The ideal antibody for immunoaffinity purification is one that has a high affinity for the antigen and whose binding can be reversed by a simple but gentle change in pH.

Immunoaffinity purification was performed in three steps: preparation of the antibody column, the binding of antigen to the antibody-bead matrix, and the elution of the antigen from the column.

2.9.1 ANTIBODY FOR IMMUNOAFFINITY PURIFICATION

The anti-HLA-DQ IA3 (Winchester et al.) is a pan HLA-DQ monoclonal antibody and was kindly provided by Dr Robert Winchester (Columbia University, New York, NY).

2.9.2 TECHNIQUE OF AFFINITY CHROMATOGRAPHY

Crude membrane fractions of the cell lines were prepared by hypotonic lysis and differential centrifugation. After washing in ice-cold phosphate buffered saline (PBS) , 10g of cell pellet were lysed in PBS with 3% nonidet-P40 (NP40), 1 μ g/ml leupeptin, 1 μ g/ml pepstatin and 5mM ethylenediaminetetraacetic acid (EDTA). Cell lysates were cleared for nuclei and debris by centrifugation at 100,000 x g for 90 minutes at 4°C.

Immunoaffinity chromatography columns of anti-DQ IA3-Cyanogen Bromide-activated sepharose (Pharmacia) were prepared²⁹³. The mAb IA-3 was mixed with the CNBr-activated sepharose beads and incubated at room temperature with gentle rocking overnight. The beads were washed twice with 0.5M sodium phosphate (pH 7.5) and once with 1M

NaCl, 0.05M sodium phosphate (pH 7.5). 10 volumes of 100mM ethanolamine (pH 7.5) was added and incubated overnight with gentle mixing. The beads were further washed twice with PBS, 0.01% merthiolate was added and they were stored at 4°C until used.

The detergent soluble membrane fractions from the cell lines were passed over a pre-column of Sepharose CL 4B (pharmacia) followed by passage over the affinity column with cyanogen bromide-activated Sepharose beads linked to the anti-DQ IA-3. After the lysates have been passed over the columns, the columns were washed extensively and then eluted with 0.05M diethylamine (pH = 11.5). The DQ molecules were immediately neutralized with 1M Tris (pH 6.8) and concentrated by ultrafiltration (Centriprep; Amicon, Beverley, MA). 50µl aliquots of eluates were analyzed by 12% SDS-PAGE and silver staining to confirm protein purity (Fig 5.1).

2.10 PEPTIDE ELUTION

The HLA DQ eluates obtained after immunoaffinity purification were concentrated on a CENTRICON-10 Microconcentrator (Amicon, Beverly, MA). The centricon tube was washed in 0.1% tri-fluoroacetic acid (TFA) for 1 hour. The tube was filled with 1 ml of TFA, the eluate was added and centrifuged at 5000 x g for 1 hour to obtain an ultrafiltrate. 1ml of TFA was added and centrifugation performed for 1 hour to obtain another ultrafiltrate stored in a different tube. This step was repeated to obtain more ultrafiltrate. The ultrafiltrates containing HLA-DQ bound peptides were stored at -70°C until characterization.

2.11 SEPARATION OF PEPTIDES: REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (r-HPLC)

The reversed-phase (RP) HPLC separation of any peptide or protein mixture is dependent upon the strength of the hydrophobic interactions of each component in the mixture with

the hydrophobic surface of the column matrix and the elution strength of the organic solvent in the mobile phase. When peptides or protein mixtures are applied to a RP column, the adsorbed peptides or proteins are eluted in order of least to most strongly bound molecules by increasing the organic solvent concentration in the elution buffer, collected as individual chromatographic fractions, and analyzed separately.

Organic solvent (acetonitrile) was removed from the RP column with degassed, HPLC-grade water using a gradient from 100% organic solvent to 100% water over 15 minutes at 1ml/min. The RP column was then equilibrated by pumping 100% trifluoroacetic acid (TFA)/acetonitrile buffer at 1ml/min. This was gradually switched to 100% TFA buffer with a 10 to 15 min linear gradient and equilibrated at 100% TFA buffer for an additional 20 minutes. Equilibration was achieved when the pressure and detector absorbance were constant. A blank run without any injection for the equilibrated column was made. This was by pumping at 1ml/min, a linear gradient from 0 to 100% TFA/acetonitrile buffer over 45 min at isocratic conditions, at 100% TFA/acetonitrile buffer for 5 min, returned to 100% TFA buffer for 15 min (total run time from gradient start to completion of reequilibration was 80min). Detection settings was 0.1 absorption units full scale (AUFS) at ~210 to 220nm for 50 to 200pmol peptide.

The DQB1*0301 and DQB1*0501 eluted peptides and the RP peptide standard (transferrin), were centrifuged at 5000xg for 5 min. An aliquot of each solution was withdrawn into an HPLC syringe that was rinsed with TFA buffer through a needle compatible with the HPLC injector. The injection loop was loaded with 10µl of the peptide. The HLA DQB1*0301 and DQB1*0501 peptides were separated by HPLC on a Vydac microbore C18 reversed phase chromatography (RPC) column (250X2.1mm; 300Å; 5µm). Chromatographic analysis was monitored at multiple UV wavelengths simultaneously.

2.12 PEPTIDE SEQUENCING

Peptide sequencing was performed at the HHMI Biopolymer and W. M. Keck Foundation Biotechnology Resource Laboratory at the Yale University School of Medicine, New Haven, Connecticut, USA.

2.12.1 EDMAN DEGRADATION

The chemical process employed by automated protein/peptide sequencers is derived from the technique originated by Edman in the 1950s for the sequential degradation of peptide chains²⁹⁸⁻²⁹⁹. The first step in this degradation is the selective coupling of a peptide's amino-terminal amino acid with the Edman reagent, phenylisothiocyanate (PITC), a reaction catalyzed by an organic base delivered with the coupling reagent. The second step is cleavage of this derived amino acid from the remainder of the peptide, a reaction accomplished by treating the peptide with a strong organic acid. Each repeated coupling/cleavage cycle occurs at the newly formed amino-terminal amino acid left by the previous cycle. These repetitive cycles provide sequential separation of the amino acids which form the primary structure of the peptide.

The identity of the amino acid removed by Edman degradation is determined by converting the cleaved amino acid derivative (anilinothiazolinone, ATZ) to the more stable derivative (phenylthiohydantoin, PTH) (Fig 2.5). In modern sequencers, this conversion is accomplished automatically, using an aqueous solution of a strong organic acid, in a reaction vessel separate from that in which the Edman degradation occurs. The PTHs produced from each degradation cycle are then transferred directly and automatically from the sequencer conversion vessel to an on-line analysis system.

For pool sequencing of peptides bound to HLA DQB1*0301 and DQB1*0501, dominant peaks identified by HPLC were first removed, then the remaining fractions were pooled,

speedvaced to dryness and redissolved in 0.05% TFA and 50% acetonitrile. The sample was then subjected to 17 cycles of Edman degradation using an Applied Biosystems (Foster City, CA) 477A pulsed liquid protein sequencer equipped with on-line HPLC. Cysteine was not quantitated. The pool sequence data are shown in tables 5.1 and 5.2. From the analysis of the pool sequence, a motif for peptide binding to HLA DQB1*0301 and HLA DQB1*0501 were derived. The source proteins of peptides were identified by searching the Protein Identification Resource (PIR), Genpept and Swiss Protein Databases.

2.13 SOLUTIONS AND REAGENTS

Blocking Solution: 500ml

| | |
|-----------------------|-----------------|
| 4X SSPE | 100ml 20X stock |
| 0.1% Lauroylsarcosine | 5ml 10% stock |
| 1.0% Blocking Reagent | 5g |
| dH ₂ O | 390ml |

Blocking reagent from Boehringer Cat No 1096 176

Lauroylsarcosine Sigma L-5125

2X SSPE/0.1% SDS: 500ml

| | | |
|----------|-------|-----------|
| 2X SSPE | 50ml | 20X Stock |
| 0.1% SDS | 2.5ml | 20X Stock |

TMACl Wash Solution: 500ml

| | | |
|-----------|-------|--------------------|
| 3M TMACl | 300ml | 5M Stock |
| 50mM Tris | 25ml | 1M Tris/HCl (PH 8) |

| | | |
|----------|-------|---------------|
| 0.1% SDS | 2.5ml | 20% Stock |
| 2mMEDTA | 2ml | 0.5M (PH 8) |

Buffer 1 : 10 litres of 10X Stock

| | |
|----------------------------------|--------|
| 1M Tris | 1211g |
| 1.5MNaCl | 876.6g |
| pH solution to 7.5 with conc HCl | |

Dilute 1/10 before use.

Buffer 2: 500ml

| | | |
|---------------------|-------|-------------------|
| 0.1M Tris | 50ml | 1M Stock (pH 7.5) |
| 0.15M Nacl | 75ml | 1M Stock |
| 1% Blocking reagent | 5g | |
| dH ₂ O | 370ml | |

Buffer 3: 500ml

| | | |
|------------------------|------|----------------------|
| 0.1M Tris | 50ml | 1M Tris/HCl (pH 9.5) |
| 0.1M Nacl | 50ml | 1M Stock |
| 50mM MgCl ₂ | 25ml | 1M Stock |

CSPPD (Lumigen) Solution : 100ml

1ml of CSPPD is added 100ml of filtered buffer 3. The container is wrapped in tin foil and stored at 4°C.

Boehringer

Anti-digoxigenin-AP, Fab fragments:

2.14 DNA CONTROL KIT

DRB1 and DQB DNA control kits of the British Society for Haematology and Immunology (BISHI) were obtained from the United Kingdom Transplant Support Service Authority, Bristol.

2.15 CHEMICAL AND MATERIAL SUPPLIERS

AMERSHAM INTERNATIONAL PLC

Amersham place, Little Chalfont, Amersham, Buckinghamshire
HP7 9NA.

BDH

Merck Ltd., Merck House, Poole, Dorset, BH15 1TD.

BIO-RAD LABORATORIES LTD.

Bio-Rad House, Maylands Avenue, Hemel Hempstead,
Hertfordshire, HP2 7TD.

BOEHRINGER MANNHEIM UK (DIAGNOSTICS AND BIOCHEMICALS) LTD.

Bell Lane, Lewes, East Sussex, BN1 1LG.

DIFCO LABORATORIES LTD.

P. O. Box 14B, Central Avenue, East Molesey, Surrey, KT8 0SE.

DUPONT (UK) LTD.

Diagnostics and Biotechnology Systems, Wedgwood Way,
Stevenage, Hertfordshire, SG1 4QN.

GIBCO BRL

Life Technologies Limited, Unit 4, Cowley Mill Trading Estate,
Longbridge Way, Uxbridge, UB8 2YG.

FLUKA CHEMIKA-BIOCHEMIKA

Fluka Chemicals Ltd., The Old Brickyard, New Road, Gillingham,

Dorset, SP8 4JL.

FMC BIOPRODUCTS

Flowgen Instruments Ltd., Broad Oak Enterprise Village, Broad Oak Road,
Sittingbourne, Kent, ME9 8AQ.

IBI

International Biotechnologies Inc., 36 Clifton Road,
Cambridge, CB1 4ZR.

ICN BIOCHEMICALS

Division of ICN Biomedicals Inc., Cleveland, OH 44128.

NBS BIOLOGICALS

New Brunswick Scientific (UK) Ltd., Edison House,
163 Dixons Hill Road, North Mymms, Hatfield, AL9 7JE.

NEW ENGLAND BIOLABS

CP Laboratories, P. O. Box 22, Bishop's Stortford,
Herts, CM23 3DX.

PHARMACIA LKB

Pharmacia Biosystems Limited, Biotechnology Division, Davy Avenue,
Knowlhill, Milton Keynes, MK5 8PH.

STRATAGENE CLONING SYSTEMS

Stratagene Ltd., 140 Cambridge Innovation Centre, Cambridge Science Park/Milton Road,
Cambridge, CB4 4GF.

SIGMA CHEMICAL COMPANY

Fancy Road, Poole, Dorset, BH17 7NH.

UNITED STATES BIOCHEMICAL CORPORATION

Cambridge Bioscience, 25 Signet Court, Newmarket Road,
Cambridge, CB5 8LA.

| Type | Primer | Location (nt) and product size |
|--------|--|--------------------------------|
| HPV 16 | Sense: 5'-AAGGCCAACTAAATGTCAC-3' | 7763-7781 |
| | Antisense: 5'-(GCGGATCC)TGCTGCTTTTATACTAA-3' | 78-61 |
| | (Seedorf et al, 1985) ²⁷⁸ | (+5'BamHI site) 228bp |
| HPV 18 | Sense: 5'-CACGGCGACCCTACAAGCTACCTG-3' | 127-150 |
| | Antisense: 5'-TGCAGCACGAATGGCACTGGCCTC-3' | 531-508 |
| | (Coles & Danos, 1987) ²⁷⁹ | 405bp |
| HPV 31 | Sense: 5'-AGAAAGACCTCGGAAATTG-3' | 125-143 |
| | Antisense: 5'-TACCTCTGTTTCTGTTAAC-3' | 233-215 |
| | (Goldsborough et al., 1989) ²⁸⁰ | 109bp |
| HPV 33 | Sense: 5'-CTACAGTGCGTGGAATGCAAAAAACC-3' | 190-215 |
| | Antisense: 5'-CGGGACCTCCAACACGCCGCAC-3' | 536-515 |
| | (Cole & Streeck, 1986) ²⁸¹ | 347bp |

Table 2.1: Type Specific Primers used for HPV amplification and annealing temperatures

| HLA Allele | Primer sequences FAMP/RAMP | Size/ PCR product. |
|-----------------|---|--------------------|
| DQB1*0201 | 5' GTGCGTCTTGTGAGCAGAAG 3' 5' GCAAGG TCGTGCCGAGCT 3' | 205bp |
| DQB1*0201/ 0302 | 5' GACGGAGCGCGTGCGTCT 3' 5' CTGTTCCAGTACTCGGCCG 3' | 129bp |
| DQB1*0301/ 0304 | 5' GACGGAGCGCGTGCGTTA 3' 5' AGTACTCGGCGTCAGGCG 3' | 122bp |
| DQB1*0302/ 0303 | 5' GACGGAGCGCGTGCGTTA 3' 5' AGTACTCGGCGTCAGGCG 3' | 122bp |
| DQB1*0303 | 5' GACGGAGCGCGTGCGTTA 3' 5' CTGTTCCAGTACTCGGCCG 3' | 129bp |
| DQB1*0601 | 5' GCCATGTGCTACTTCACCAAT 3' 5' CACCGTGTCCAACCTCCGCT 3' | 198bp |
| DQB1*0601/ 0301 | 5' GACGGAGCGCGTGCGTTA 3' 5' CTGTTCCAGTACTCGGCCG 3' | 129bp |
| DQB1*0304 | 5' GACGGAGCGCGTGCGTTA 3' 5' CTGTTCCAGTACTCGGCCG 3' | 129bp |

Table 2.2: Sequence specific primer pairs for typing the HLA DQB1*03 locus.

FAMP Forward amplification primer; RAMP reverse amplification primer

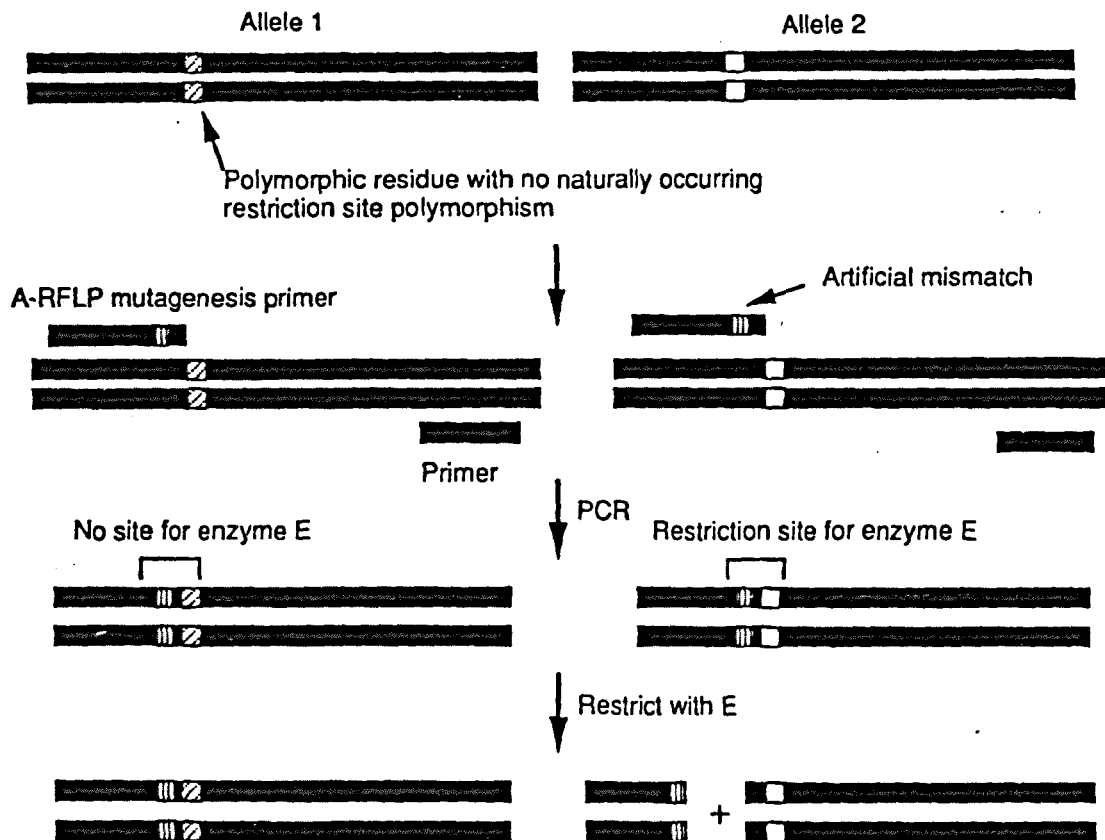


Fig 2.1: Principles of A-RFLP

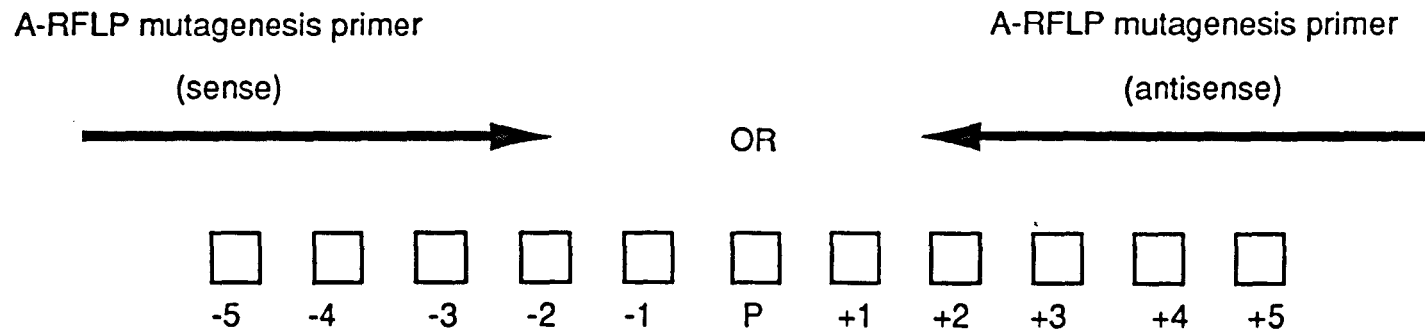
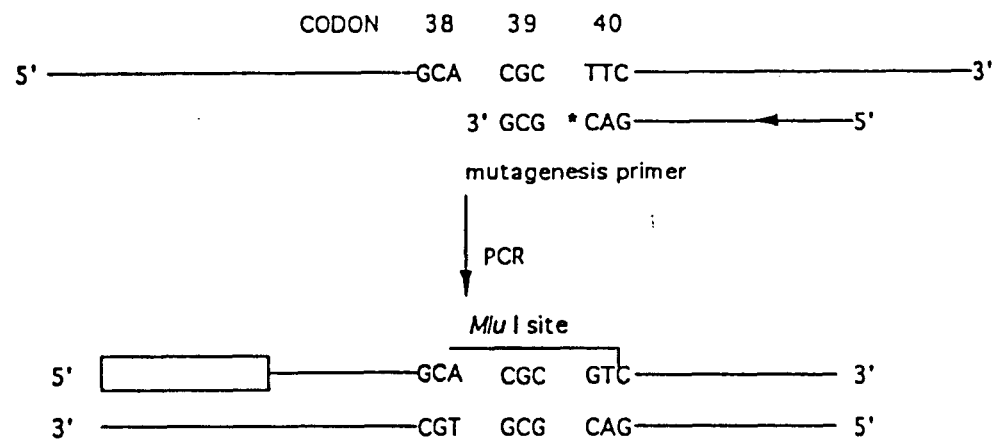


Fig 2.2: The design of A-RFLP Primers

DQB1*03 Alleles



Non-DQB1*03 Alleles

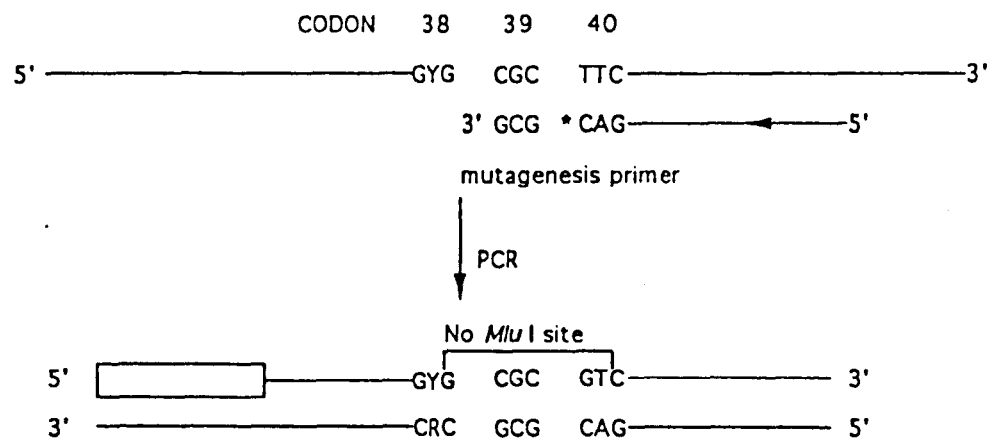


Fig 2.3: A-RFLP for HLA DQB1*03

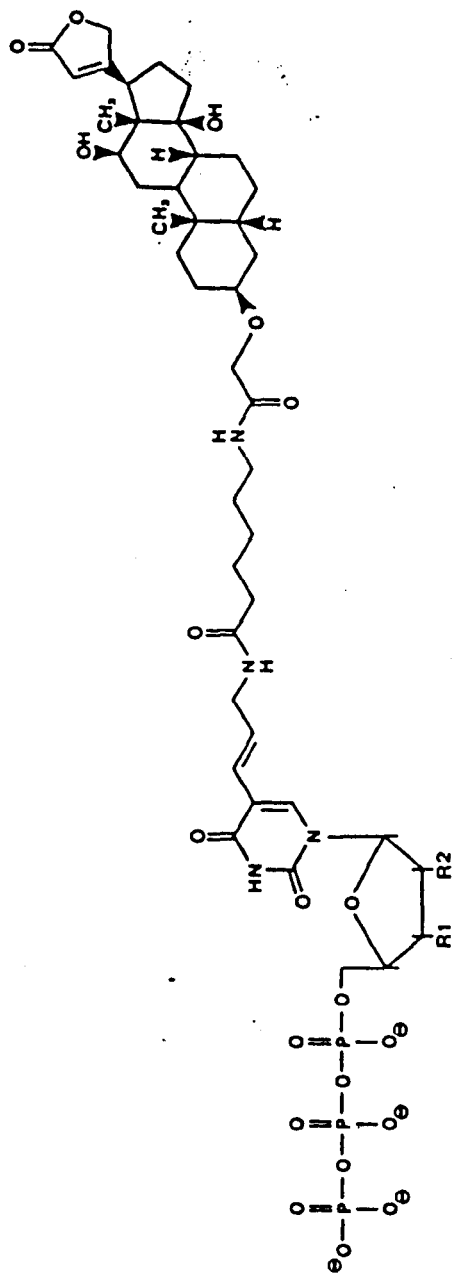


Fig 2.4: Digoxigenin-UTP (R1 = OH, R2 = OH)/Digoxigenin-dUTP (R1 = OH, R2 = H)/Digoxigenin-ddUTP (R1 = H, R2 = H)

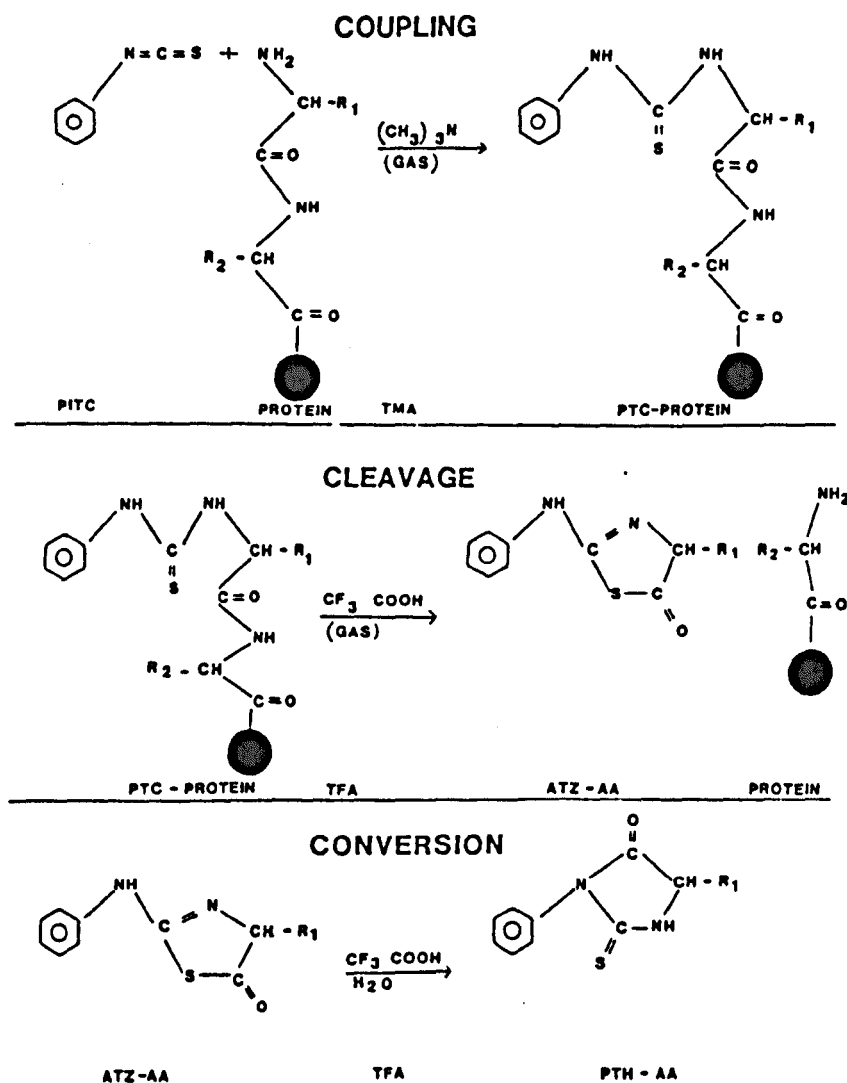


Fig 2.5: Schematic illustration of the principles of Edman chemistry showing the coupling, cleavage and conversion steps.

CHAPTER 3: ASSOCIATION BETWEEN HLA DQB1*03 AND CERVICAL INTRA-EPITHELIAL NEOPLASIA

3.1 INTRODUCTION

3.2 RESULTS

3.3 OVERALL RESULTS (APPENDIX 1)

3.4 ASSOCIATION BETWEEN HLA DQB1*03 AND CIN

3.5 ASSOCIATION BETWEEN HLA DQB1*03 AND HPV

3.6 SUMMARY AND DISCUSSION

3.1 INTRODUCTION

Recently, Wank & Thomssen²⁵⁵ showed a significant association between HLA DQB1*03 and cervical cancer. Subsequent reports have not consistently confirmed this observation (reviewed in chapter 1 and Odunsi & Ganesan³⁰⁰). Evidently there is heterogeneity within results depending on the size and type of population examined and techniques used for the HLA and HPV typing. This chapter reports the results of HPV and HLA DQB1*03 typing conducted in a Caucasian population.

Allelic products of the polymorphic DQA1 and DQB1 genes encode functional DQ molecules through *cis*- and *trans*-complementations. Cis-dimers comprise α and β chains encoded by DQA1 and DQB1 genes of the same chromosome, and trans-dimers are encoded by genes on homologous chromosomes. Although it is clear that HLA-DP, -DQ and -DR products can all present antigen to human CD4+ T cells, HLA-DR restriction overwhelmingly predominates. The apparent inefficiency of HLA-DQ as an antigen restriction molecule presents a perplexing paradox: HLA-DQ restricted T cell clones are rare, reflecting the low expression of the dimer on antigen presenting cells, yet disease association studies relatively frequently implicate HLA-DQ, rather than -DR alleles in predisposition to autoimmune and some infectious diseases. Human T-cell clones so far characterized show a marked bias against HLA-DQ restriction, reflecting the low level of expression on APCs in the periphery. Although this low frequency of DQ-restricted clones may reflect a truly marginal role in the immune response, the stimulation requirements or effector functions of DQ-restricted clones may differ from those in conventional studies.

3.2 RESULTS

The ARFLP-PCR technique on DNA from cervical smears, following *Mlu* I digestion, can lead to three possible results: negative for DQB1*03, heterozygous or homozygous for DQB1*03 (Fig 3.1).

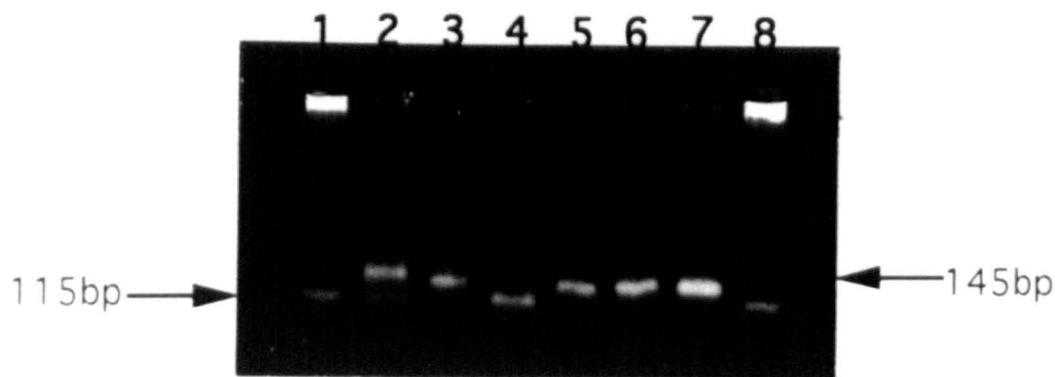


Fig 3.1: A 4% metaphor agarose gel showing amplified DNA after PCR with primers A and B with and without digestion by *MluI*

The size of amplified DNA is 145bp and on digestion with *MluI*, a 115 and 30bp product is produced in DQB1*03 homozygotes. DNA for all controls were from the British Society for Histocompatibility and Immunogenetics. Arrows show the 145 and 115bp products. Lanes 2 and 3 show heterozygous DQB1*03 control with and without digestion by *MluI*. Lanes 4 and 5 show homozygous DQB1*03 control with and without digestion with *MluI*. Lanes 6 and 7 show non-DQB1*03 control with and without digestion with *MluI*. Lanes 1 and 8 are 123 bp markers.

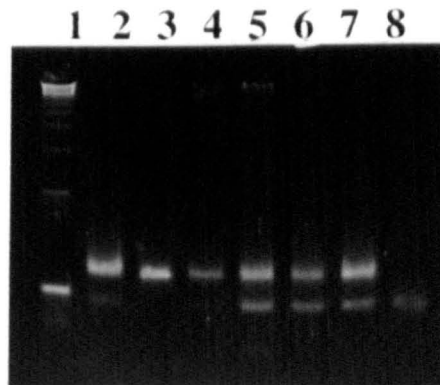


Fig 3.2: Example of A-RFLP on study samples. Lane 1: 123bp ladder DNA; Lane 2: JRA 28, DQB1*03 heterozygous cell line after digestion; Lane 3: JRA 28, DQB1*03 heterozygous cell line before digestion; Lane 4: Amai, Non DQB1*03 cell line after digestion; Lanes 5-8: samples from patients with CIN- 5: heterozygous DQB1*03; 6: Heterozygous DQB1*03; 7: Heterozygous DQB1*03; 8: Homozygous DQB1*03.

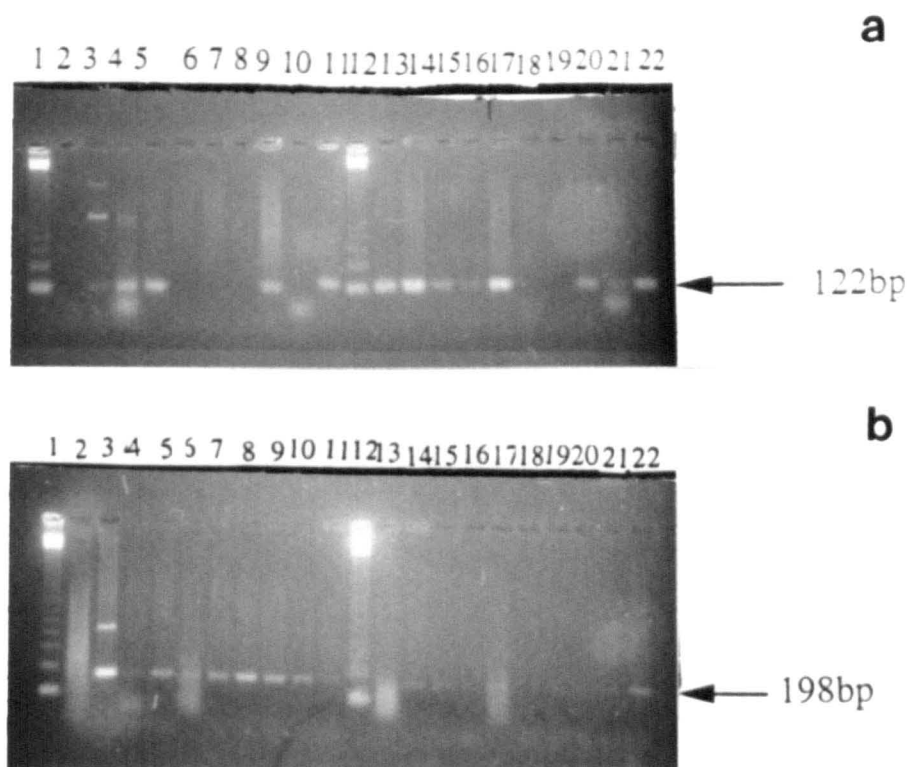


Fig 3.3: PCR products obtained by HLA DQB1*03 sequence specific primers. (a) 122 bp product obtained with primer pair PB5'09 and PB3'09 to identify DQB1*0301/0304. Lanes 1 and 12: 123 bp marker; Lanes 2 and 18: negative control; Lane 3: positive control DNA. An internal amplification control primer pair PC'5 and PC'3 (amplifies the third intron of DRB1 genes) was included in this reaction to give a 796 bp fragment; the rest represent study samples. (b) 198 bp product obtained on the same set of samples with primer pair PB5'03 and PB3'04 to identify DQB1*0601. Lanes 1 and 12: 123bp marker; Lanes 2 and 18 are negative controls; Lane 3: positive control DNA and the remaining lanes are study samples. Fig. for DQB1*0304 not shown since all were negative for this set of primers. Allelic assignment was by comparing and integrating positive results.

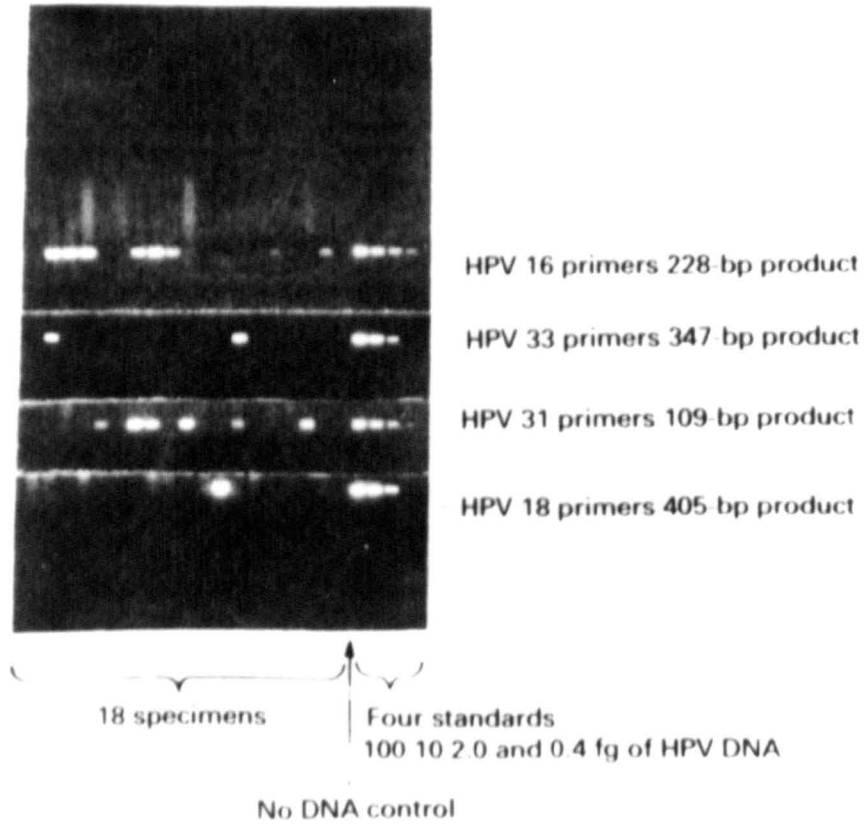


Fig 3.4: Type specific amplification of HPV.

3.3 OVERALL RESULTS

HLA DQB1*03 typing was performed on DNA from cervical smears of 178 women with CIN (CIN I = 66; CIN III = 112) and 420 healthy women who had a normal smear. All samples were successfully amplified for the locus. HPV typing was performed for types 16, 18, 31 and 33 on all the test and control samples. The HLA DQB1*03 and HPV results on individual samples are included in the tables showing the complete HLA DQ-DR typing results (Refer to Appendix 1). As shown in appendix 1, the women are either homozygous for the HLA DQB1*03 alleles (0301, 0302, 0303 and 0304) or heterozygous (DQB1*03 allele in combination with any other DQB allele). The analysis of HLA DQB1*03 is presented first.

Table 3.1 is a summary of the distribution of HLA DQB1*03. Of CIN cases, 61% were positive (56% of CIN I, 64% of CIN III) for the HLA DQB1*03 type, compared to 34% of controls. The association was significant (χ^2 trend = 37.3, $p < 0.001$), and the odds ratio for CIN overall was 3.03 (95% CI 2.11-4.35). The association was significant for both CIN III (odds ratio 3.45 vs 2.45) and CIN I, stronger for CINIII, but not significantly different from CIN I.

One hundred and thirty-one patients with CIN (73.5%) were positive for one or more HPV types 16, 18, 31, 33. Of HPV-positive CIN, 64% were of the type DQB1*03. There was a significant association between DQB1*03 and HPV (χ^2 trend = 38.6, $p < 0.001$) with an odds ratio of 3.43 (95% CI 2.28-5.15).

3.4 ASSOCIATION BETWEEN HLA DQB1*03 AND CIN (Tables 3.2 and 3.3)

Of women with CIN, 38% were negative for DQB1*03, while 37% were heterozygous and 23% homozygous for the DQB1*03 locus (χ^2 trend = 39.01, $p < 0.001$). Compared

with controls, the odds ratio was greater for homozygosity (4.0, 95% CI 2.43-6.6) than for heterozygosity (2.63, 95% CI 1.75-3.94). Further typing of the DQB1*03 locus in positive samples by PCR-SSP showed that the 0301 allele that was present in 40% of CIN as opposed to 9% of controls (odds ratio 2.53, 95% CI 1.79-3.57; χ^2 trend =28.6, $p<0.001$). DQB1*0302 was present in 32% and 10% of CIN and controls respectively (odds ratio 1.84, 95% CI 1.29-2.62). The association between HLA DQB1*03 and CIN is shown in table 3.2 while association between DQB1*03 alleles and CIN is shown in table 3.3.

3.5 ASSOCIATION BETWEEN HLA DQB1*03 AND HPV (Tables 3.4, 3.5 and 3.6)

HPV typing was performed for the major oncogenic types, HPV 16,18, 31 and 33. Of CIN cases, 57% were positive for HPV 16, 7% for HPV 18, 12% for HPV 31 and 7% for HPV 33 and 16% were positive for multiple types. All types correlated strongly with DQB1*03 but there was insufficient data to find a difference between the types. The highest odd ratio was found for women with HPV 18 or multiple types. There was a significant correlation with "gene dosage" at the DQB1*03 locus, with 39% of HPV positive CIN being heterozygous and 24% homozygous for DQB1*03 (χ^2 trend =37.9, $p<0.001$). Homozygosity was significantly associated with HPV positive CIN (odds ratio 4.47, 95% CI 2.58-7.77). Further typing of the HLA DQB1*03 locus in positive samples showed that the 0301 allele was most strongly associated with HPV infection (odds ratio 2.69, 95% CI 1.88-3.94; χ^2 trend=32.9, $p<0.001$). Table 3.4 shows the association between HLA DQB1*03 and HPV type while table 3.5 shows the effect of zygosity at the DQB1*03 locus. Table 3.6 shows the association between individual DQB1*03 alleles and HPV.

SUMMARY AND DISCUSSION

Cervical cancer and CIN have been shown to be strongly associated with the oncogenic types of the human papillomaviruses (16,18,31,33,35,39,45,51,52,56 and 58) in several cross-sectional studies^{68,85}. However, additional factors must operate to determine the progression from normal epithelium to CIN and cervical cancer after HPV infection. One host factor is possibly immunological, as in other virus induced cancers, such as nasopharyngeal carcinoma due to Epstein-Barr virus³⁰¹. In cervical disease this is supported by the fact that spontaneous regression of low grade CIN is frequently observed. Thus immunological mechanisms, in particular the cellular immune response, may play a significant role in the development of CIN and cervical cancer after HPV infection.

To address these issues this large study, of sufficient test samples and controls evaluates the significance of DQB1*03 association with cervical intra-epithelial neoplasia. This study was performed in CIN, as it is the precursor lesion of cervical cancer, and the results of HLA typing may be relevant particularly when correlated with the HPV status. Further it is quite important to evaluate the association between CIN, HPV and DQB1*03 using controls that are negative for HPV and have a normal cytology. The interpretation and reporting of negative HPV results must be interpreted in the context of the detection system used. In this study, HPV 16, 18, 31 and 33 were tested for and a negative result simply means that the specimen does not contain any of these HPV types.

The use of consensus primers in PCR for HPV can result in competition between non-specifically primed human DNA with HPV DNA and between different types of HPV DNA in individual clinical specimens, and the apparent level of any particular type may be distorted after amplification. For this reason, type specific primers were used in this study.

Consensus primers are reserved for qualitative demonstration of the presence of HPV types other than those specific types tested for.

The typing for HLA DQB1*03 was performed with a rapid technique which was concordant with data based on sequencing³⁰². The advantage of this method lies in the need for a single mutagenic primer, which is used in a single step PCR amplification. This technique is also informative in assessing whether the individual sample is heterozygous or homozygous for the DQB1*03 locus. Likewise, the PCR-SSP technique is an accurate and rapid technique for detecting genetic variability with a high degree of resolution. Each primer pair identifies two cis-located sequence motifs, which allows the separation of all homozygous and heterozygous combinations of DQB1*03. For instance, a DQB1*0301/DQB1*0302 cannot be distinguished by PCR-SSO typing. However, the two alleles can be unequivocally assigned by the PCR-SSP technique. Since the method is ideal for analyzing a small number of samples, it was not used for typing the remaining DQB alleles.

The results show a significant association between CIN and DQB1*03 that is only slightly stronger for CIN III than CIN I. The association between CIN and DQB1*03 that was found (odds ratio 3.03) was less strong than that reported by Wank and Thomssen, but a slightly stronger association in HPV positive CIN (odds ratio 3.43) was observed than that reported by Van den velde et al³⁰³. Homozygosity at DQB1*03, was significantly associated (odds ratio 4.0) with CIN and was more strongly related than heterozygosity, a result not reported so far in any previous studies. The 0301 allele was the most strongly associated with CIN (odds ratio 2.53, $\chi^2 = 28.6$, $p < 0.001$) but 0302 was also positively related. This agrees with Wank and Thomssen's DNA typing data for 0301 on their original sample of cervical cancer patients²⁵⁷.

A significant association with HPV positive CIN and DQB1*03 was found for all HPV types tested (16,18,31,33). Again homozygosity at the DQB1*03 was strongly associated with HPV positive CIN (odds ratio 4.47) with intermediate risk found for heterozygotes. Typing for HPV has not been uniformly performed in all the previous studies, but in general HPV positive CIN was significantly associated with the DQB1*03 phenotype. In this study type specific primers for the major oncogenic types of HPV were used and it is possible that some of the HPV negative CIN are positive for other types. Detailed typing for other HPV types is only likely to increase the strength of the association. The results also show that the association between DQB1*03 and HPV positive CIN is intermediate in risk for CIN I and greater for CIN III, in agreement with the natural history of the disease. These results suggest that probably the DQB1*03 locus may be an important determinant in allowing the HPV infection to be tolerated and permit the progression to CIN or cancer.

Another disease due to HPV infection, recurrent respiratory papillomatosis, has been shown to be associated with the DQB1*03 phenotype³⁰⁴. In an analysis of 16 patients, 75% were positive for DQB1*03. Analysis of HLA class I and II using restriction fragment length polymorphisms, in New Zealand rabbits infected with Shope cotton-tail rabbit papillomavirus, showed a strong linkage between wart regressions and DR locus, and an increased risk of malignant transformation with the DQ locus³⁰⁵. Thus based on this study and others, the DQB1*03 locus seems to be important for HPV associated disease. The results of the analysis of HLA DR and DQ in squamous cell carcinoma reported by Apple et al³⁰⁶ in a Hispanic population showed no significant association with the DQB1*03 locus, although the haplotype DRB1*0407-DQB1*0302 was associated with increased risk of cervical carcinoma.

In summary, it is possible that women who are positive for the DQB1*03 phenotype may be unable to mount an effective cytotoxic T cell response against HPV infection. This is

particularly important as it has been shown that HPV16 E7 is a target for cytotoxic T cells and to mediate tumour rejection³⁰⁷.

Table 3.1: Summary of Distribution of HLA DQB1*03

| Patients (No.) | HLA DQB1*03 (Positive) % | Odds Ratio (95% CI) |
|----------------------------------|-------------------------------------|--------------------------------|
| CIN (178) | 109 (61) | 3.03 (2.11-4.35) |
| CIN 1 (66) | 37 (56) | 2.45 (1.45-4.12) |
| CIN 3 (112) | 72 (64) | 3.45 (2.23-5.33) |
| HPV negative CIN (47) | 25 (53) | 2.18 (1.19-3.97) |
| HPV positive CIN (131) | 84 (64) | 3.43 (2.28-5.15) |
| Controls (420) (HPV negative) | 144 (34) | 1* |

χ^2 (trend) for (controls, CIN 1 and CIN 3) =37.3, $p < 0.001$.

χ^2 (trend) for controls, HPV negative and HPV positive) = 38.6, $p < 0.001$.

* reference category

Table 3.2: Association between HLA DQB1*03 and CIN

| HLA | Controls (%) | CIN (%) | Odds Ratio (95% CI) |
|-----------------------------|-----------------|------------|------------------------|
| non DQB1*03 | 276 (65) | 69 (38) | 1* |
| Heterozygous for DQB1*03 | 102 (24) | 67 (37) | 2.63 (1.75-3.94) |
| Homozygous for DQB1*03 | 42 (10) | 42 (23) | 4.0 (2.43-6.60) |
| Total | 420 | 178 | |

* reference category

χ^2 (trend) = 39.01, p<0.001.

Table 3.3: Association between HLA DQB1*03 allele and CIN

| HLA DQB1*03 allele | CIN 3 (2n=224) (%) | CIN1 (2n=132) (%) | Controls (2n=840) (%) | Odds Ratio (95% CI) | χ^2 (trend) |
|-------------------------------|-----------------------------------|----------------------------------|--------------------------------------|--------------------------------|--|
| 0301 | 49 (21) | 25 (19) | 79 (9) | 2.53 (1.79-3.57) | 28.6 (p<0.001) |
| 0302 | 45 (20) | 16 (12) | 85 (10) | 1.84 (1.29-2.62) | 15.5 (p<0.001) |
| 0303 | 5 (2) | 7 (5) | 21 (2.5) | 1.36 (0.67-2.76) | 0.05 p=0.82 |
| 0304 | 0 | 0 | 1 (0.1) | 0 | |
| non DQB1*03 | 125 | 84 | 654 | 1* | |
| Total | 224 | 132 | 840 | | |

* reference category

Table 3.4: Association between HLA DQB1*03 and HPV type

| HPV Type present | Number of Patients | DQB1 *03 (positive) (%) | Odds Ratio (95% CI) |
|-----------------------------|-------------------------------|--|--------------------------------|
| 16 | 75 | 45 (60) | 2.88 (1.74-4.74) |
| 18 | 9 | 7 (77) | 6.71 (1.56-∞) |
| 31 | 16 | 11 (68) | 4.22 (1.5-11.84) |
| 33 | 10 | 6 (60) | 2.88 (0-.86-9.64) |
| Multiple types | 21 | 15 (71) | 4.79 (1.88-12.2) |
| Controls (HPV negative) | 420 | 144 (34) | 1* |

* reference category

Table 3.5: Association between HLA DQB1*03 and HPV

| HLA | Controls (%) | HPV positive CIN (%) | Odds Ratio (95% CI) |
|-----------------------------|-------------------------|-------------------------------------|--------------------------------|
| non DQB1*03 | 276 (65%) | 47 (35%) | 1* |
| Heterozygous for DQB1*03 | 102 (24%) | 52 (39%) | 2.99 (1.90-4.71) |
| Homozygous for DQB1*03 | 42 (10%) | 32 (24%) | 4.47 (2.58-7.77) |
| Total | 420 | 131 | |

* reference category

χ^2 (trend) =37.9, p<0.001.

Table 3.6: Association between HLA DQB1*03 allele and HPV

| HLA DQB1*03 allele | CIN HPV (positive) (2n=262) (%) | CIN HPV (negative) (2n=94) (%) | Controls HPV (negative) (2n= 840) (%) | Odds Ratio (95% CI) | χ^2 (trend) |
|-----------------------|---|--|---|------------------------|---------------------|
| 0301 | 60 (22) | 14 (15) | 79 (9) | 2.69 (1.88-3.94) | 32.9 (p<0.001) |
| 0302 | 45 (17) | 16 (17) | 85 (10) | 1.71 (1.17-2.50) | 10.6 (p<0.001) |
| 0303 | 9 (3) | 3 (3) | 21 (2.5) | 1.35 (0.63-2.89) | 0.71 (p<0.4) |
| 0304 | 0 | 0 | 1 (0.1) | 0 | |
| non DQB1*03 | 148 (56) | 61 (65) | 654 (78) | 1* | |
| Total | 262 | 94 | 840 | | |

* reference category

CHAPTER 4: ANALYSIS OF HLA DR-DQ ASSOCIATIONS WITH HPV AND CERVICAL INTRA-EPITHELIAL NEOPLASIA

4.1 INTRODUCTION

4.2 RESULTS

4.3 CORRELATION BETWEEN INDIVIDUAL ALLELES OF HLA DRB1, DRB3, DRB4 AND DRB5 WITH CIN

4.4 CORRELATION BETWEEN INDIVIDUAL HLA DQB1 ALLELES AND CIN

4.5 CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL HLA DQB1 AND HLA DRB ALLELES AND HPV

4.6 CORRELATION BETWEEN HLA DR/DQ HAPLOTYPES AND CIN

4.7 CORRELATION BETWEEN SIGNIFICANT HLA DR/DQ HAPLOTYPES AND HPV

4.8 CORRELATION BETWEEN SIGNIFICANT HLA DR/DQ HAPLOTYPES AND HPV TYPE

4.9 DISCUSSION

4.1: INTRODUCTION

In the preceding chapter, it was shown that there is an increased risk for HPV positive CIN in women with HLA DQB1*03, specifically DQB1*0301 (O.R. 2.53) and DQB1*0302 (O.R. 1.84) alleles. The next phase of the thesis was to perform a detailed analysis of the HLA DR and DQ alleles in patients with CIN and healthy controls in a British Caucasian population and identify haplotypes which confer both susceptibility and protection in the development of CIN after HPV infection. By defining susceptibility and protective alleles and haplotypes, these studies may help to provide a framework for understanding peptide binding and T cell recognition events in the immunological response to HPV infection. This chapter reports the detailed analysis of HLA DR-DQ in HPV associated cervical intra-epithelial neoplasia.

4.2: RESULTS

HLA DR and DQ typing was performed on DNA from cervical smears of 176 women with CIN (CIN I=63; CIN III=113) and 416 healthy women who had a normal cervical smear. All cervical samples from patients with CIN and controls were typed for DRB1, DRB3, DRB4, DRB5 and DQB1 using the PCR/SSO technique except for DQB1*03 alleles which were individually detected by allele specific primers and PCR³⁰⁸. The DR/DQ haplotypes were inferred based on known patterns of linkage disequilibrium for these loci²⁸⁶⁻²⁸⁷. HPV typing was performed on all the test and control samples and 131 of 176 (75%) cases of CIN were positive for one or more of the HPV types which were examined, and all the controls were selected to be negative for HPV. The overall results on individual samples are shown in Appendix 1. Statistical analysis was performed to evaluate for correlation between HLA type and CIN, CIN I, CIN III and HPV positive CIN.

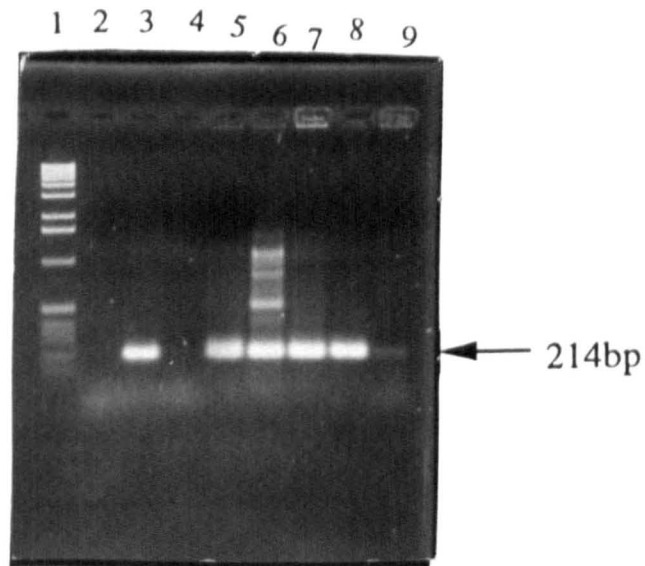


Fig. 4.1: Generic DQB1 amplification using the primer pair DQBAMP and DQRAMP to obtain a 214bp product. Lane 1: 123bp marker; Lane 2: Negative control; Lane 3: positive control DNA from cell line BVR (DQB1*0501); Lane 4: Negative control; Lanes 5 to 9: samples from patients with CIN.

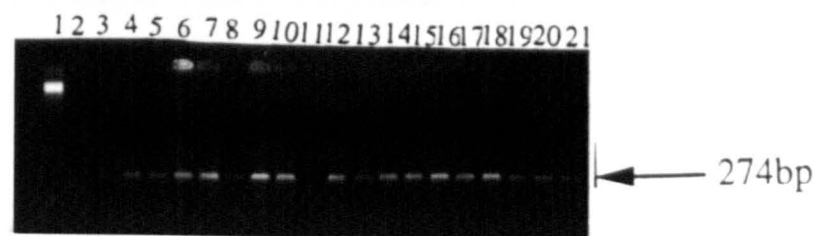


Fig. 4.2: Generic DRB1 amplification using the primer pair DRBAMP and DRRAMP to obtain a 274bp product. Lane 1: 123bp marker; Lanes 2 and 11: Negative controls; Lanes 3 and 4: positive control DNA from cell line PREISS (DRB1*0401); Lanes 5 to 10 and 12 to 21: samples from patients with CIN.

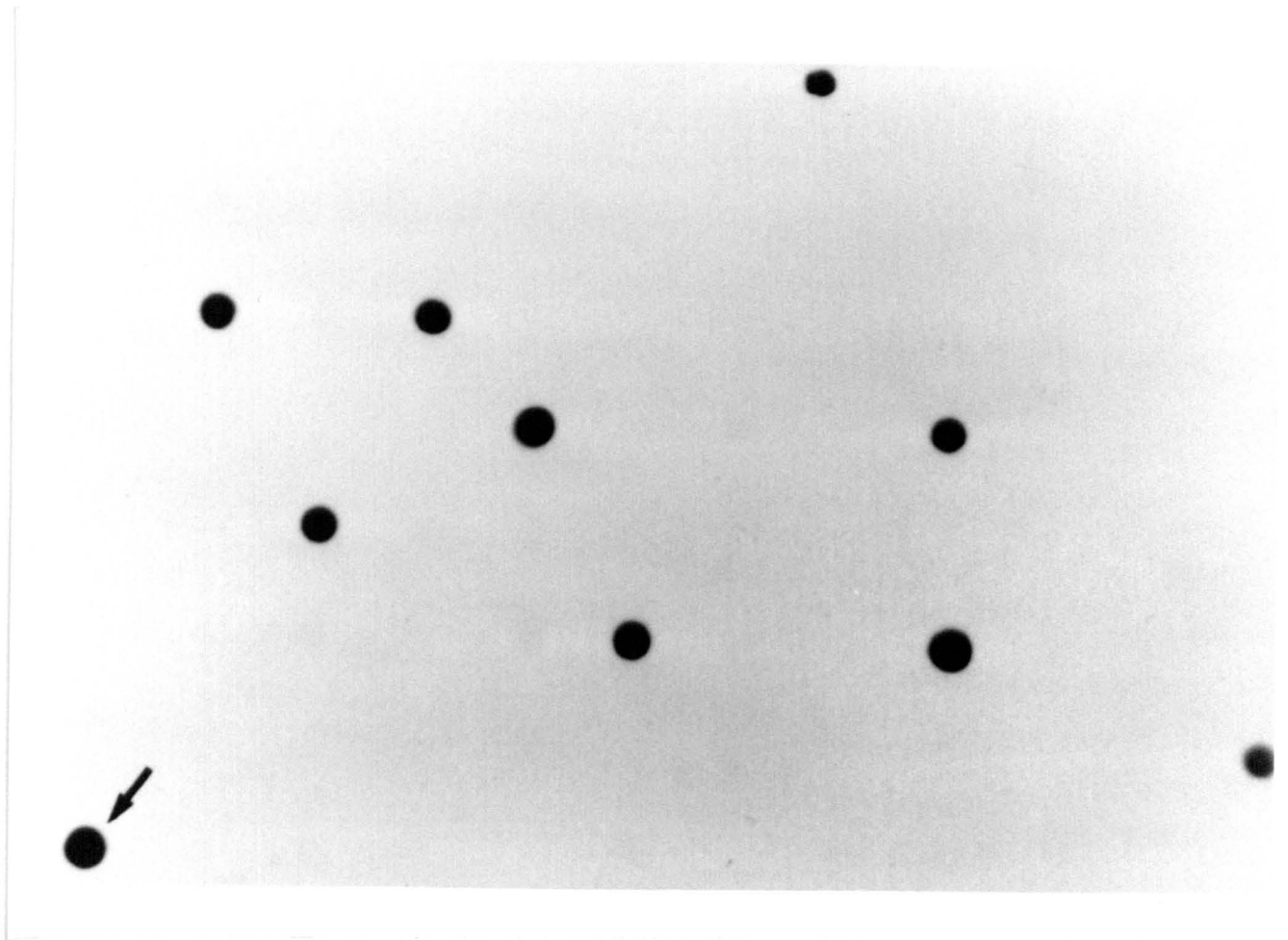


Fig 4.3: Digoxigenin labeled oligonucleotide hybridization after generic HLA DQ amplification. In this example, the probe DQB4901 identifies the HLA DQB1*0501 allele in a set of control samples. The arrow shows a positive signal from the HLA DQB1*0501 control cell line BVR, obtained from BISHI. Further confirmation of HLA DQB1*0501 in these individuals was by demonstrating positive signals from probes DQB2601 (0501, 0502, 05031, 05032) and DQB5701 (0501, 0604 and 0605).

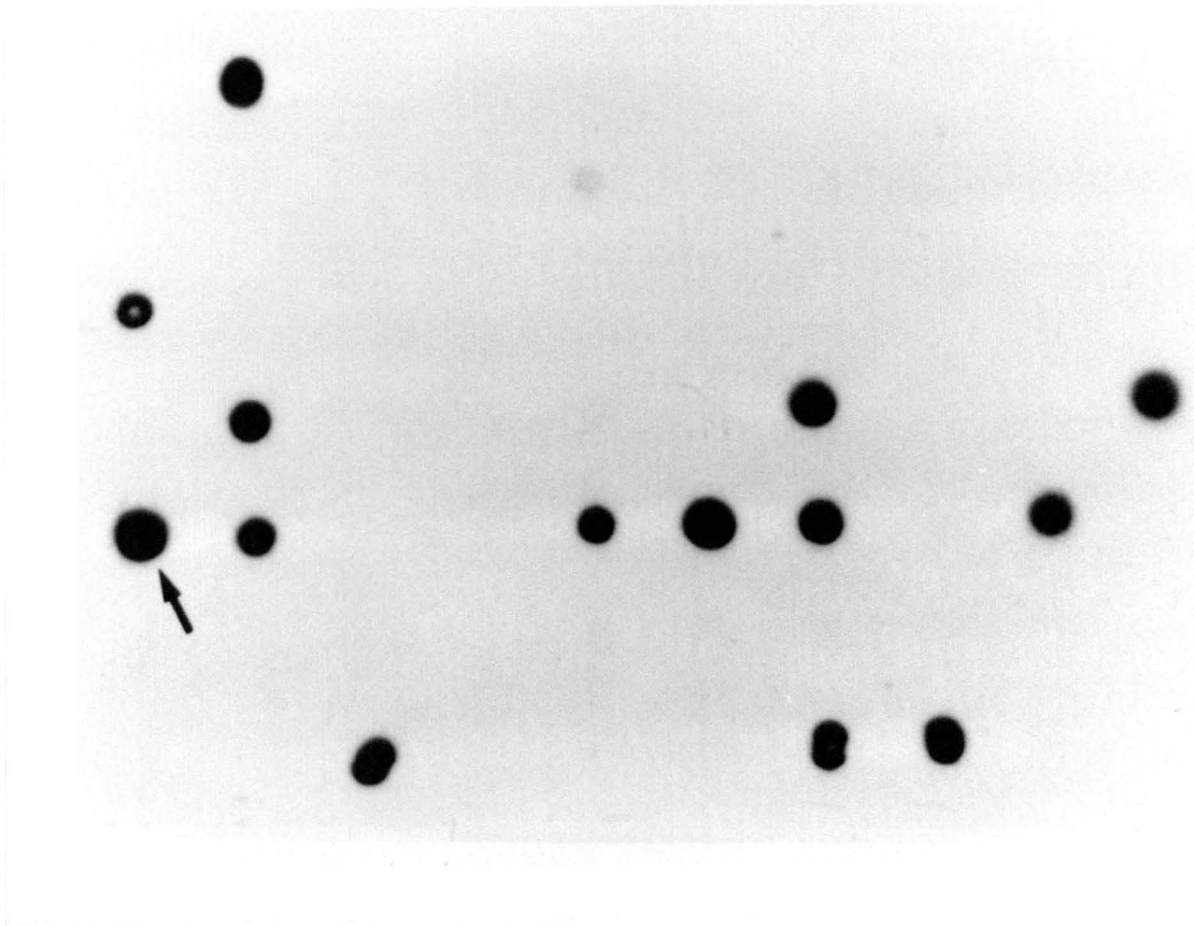


Fig 4.4: Digoxigenin labeled oligonucleotide hybridization after generic HLA DR amplification. In this example, the probe DRB1004 identifies the HLA DR4 group of alleles in a set of samples from patients with CIN. The arrow shows a positive signal from the HLA DRB1*0401 control cell line PREISS, obtained from BISHI. Further subtyping was by group specific amplification followed by DIG-labeled hybridization with the appropriate probes (section 2.5.11).

4.3 CORRELATION BETWEEN INDIVIDUAL ALLELES OF HLA DRB1, DRB3, DRB4 AND DRB5 WITH CIN (Table 4.1)

The occurrence of different DR allele groups is clearly related to CIN when analysed for heterogeneity ($\chi^2=28.76$, d.f.=12, $p=0.004$). The DR4 group correlated significantly with CIN (O.R. 1.76 {1.28-2.40}; $\chi^2=12$, $p=0.001$). Within the DR4 group there was also evidence for heterogeneity ($\chi^2=22.5$, d.f.=8, $p=0.004$). The DR4 alleles principally DRB1*0401 (O.R. 1.99, $p=0.002$); DRB1*0403 (O.R. 3.61, $p=0.02$); DRB1*0406 (O.R. 3.74, $p=0.0007$) correlated significantly with CIN. In addition, DRB1*1101 also correlated with increased susceptibility for CIN (O.R. 2.35, $p=0.004$).

There were several DR alleles which suggested a protective effect for CIN and HPV positive CIN. In particular, DRB1*0101 (O.R. 0.48, $p=0.01$); DRB1*0701/0702 (O.R. 0.58, $p=0.02$) and DRB5*0101 (O.R. 0.45, $p=0.03$) indicated a protective effect. HLA DRB1*1301 showed a protective effect for CIN III only (O.R. 0.32, $p=0.004$).

4.4 CORRELATION BETWEEN INDIVIDUAL HLA DQB1 ALLELES AND CIN (Table 4.2)

Different DQB1 alleles showed a relationship with CIN when analysed for heterogeneity ($\chi^2=49.39$, d.f.=4, $p<0.0001$). HLA DQB1*03 was the most significant, and there was no evidence of heterogeneity within it ($\chi^2=2.74$, $p=n.s.$). The DQB1*0301 demonstrated the stronger association (O.R. 2.49; $p<0.0001$), but DQB1*0302 (O.R. 1.82, $p=0.001$) was also significantly more common. Further analysis showed that the positive association with DQB1*0301 was also significantly more common in CIN I (O.R. 2.02, $P=0.01$).

Similarly, the frequency of the DQB1 alleles, DQB1*0501 (O.R. 0.48, $p=0.004$); DQB1*0402 (O.R. 0.49, $p=0.06$); DQB1*0603 (O.R. 0.47, $p=0.03$); and DQB1*0604 (O.R. 0.6, $p=0.06$) showed a protective effect with either CIN. However when the data

was re-analysed after excluding the positively associated DQ and DR alleles, none of these protective associations were significant.

The significant individual DRB1 and DQB1 alleles were analysed to assess whether homozygosity conferred an additional risk for CIN. Only at the DQB1*0301 locus could homozygosity be shown to increase risk (O.R., 4.39{1.84-10.50}; $p=0.002$). There were insufficient homozygotes of other alleles to yield clear conclusions.

4.5 CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL HLA DQB1 AND HLA DRB ALLELES AND HPV (Table 4.3)

The same alleles that were found to significantly correlate with CIN were found to correlate with HPV positive CIN. The susceptibility alleles were HLA DQB1*0301 (O.R. 2.77, $P=0.00001$); DQB1*0302 (O.R. 1.85, $P=0.003$); DRB1*0401 (O.R. 2.34, $P=0.0004$); DRB1*0403 (O.R. 3.23, $P=0.04$); DRB1*0406 (O.R. 5.05- ∞ , $P=0.0002$); and DRB1*1101 (O.R. 2.19, $P=0.02$). The alleles that showed protection to HPV positive CIN were DQB1*0501 (O.R. 0.54, $P=0.04$); DQB1*0603 (O.R. 0.44, $P=0.04$); DQB1*0604 (O.R. 0.55, $P=0.06$); DRB1*0101 (O.R. 0.56, $P=0.06$); DRB1*1301 (O.R. 0.52, $P=0.05$) and DRB5*0101 (O.R. 0.40, $P=0.03$).

4.6 CORRELATION BETWEEN HLA DR/DQ HAPLOTYPES AND CIN (Table 4.4)

The analysis for specific haplotypes was performed for all the possible DR-DQ combinations. The most common naturally occurring haplotypes (where $n \geq 10$) in British and Caucasian populations²⁸⁶⁻²⁸⁷ and ones where there was a significant correlation are displayed in Table 4.4. The two locus haplotypes DRB1*0401-DQB1*0301 (O.R. 2.22, $p=0.02$), and DRB1*1101-DQB1*0301 (O.R. 3.95, $p=0.003$) showed significantly strong associations with CIN and in particular with CIN III. Other haplotypes also demonstrated

nominally significant positive associations, but these were difficult to assess because of small numbers and multiple testing. They included haplotypes principally from the DRB1*04 group, i.e., DRB1*0401-DQB1*0302 (O.R. 1.90, $p<0.05$), DRB1*0403-DQB1*0302 (O.R. 4.34, $p=0.007$) and DRB1*0406-DQB1*0302 (O.R. 2.48- ∞ , $p=0.008$).

The only haplotype to confer a significant protective effect for CIN was DRB1*0101-DQB1*0501 (O.R. 0.48, $p=0.01$). The haplotype was also protective for CIN III (O.R. 0.37, $p=0.01$). None of the three locus haplotypes correlated positively or negatively with CIN. There were insufficient cases with homozygous DR-DQ haplotypes to analyse for correlation with risk for CIN.

4.7 CORRELATION BETWEEN THE SIGNIFICANT HLA HAPLOTYPES AND HPV (Table 4.5)

The haplotypes that correlated with CIN showed similar results for HPV positive CIN (Table 4.5). In addition, two rare haplotypes DRB1*0701-DQB1*0302 (O.R. 3.24, $p=0.03$) and DRB1*0801-DQB1*0301 (O.R. 9.63, $p=0.05$) also correlated significantly with HPV positive CIN.

4.8 CORRELATION BETWEEN SIGNIFICANT HLA DR/DQ HAPLOTYPES AND HPV TYPE (Table 4.6)

The significant susceptible and protective haplotypes were analysed to examine for correlation with individual HPV types. 131 cases of CIN were positive for one of the HPV types either alone or in combination. HPV 16 was present in 75 (57%) cases, HPV 18 in 9 (7%), HPV 31 in 17 (13%), HPV 33 in 9 (7%) and there were multiple HPV types detected in 21(16%) cases. The relation between the most significant haplotypes and specific HPV types are shown in Table 4.6. No clear association with HPV type is

apparent, but because HPV16 positive CIN was the most common it is not possible to comment on associations with other HPV types.

4.9 DISCUSSION

In this study the two DR-DQ haplotypes most clearly associated with CIN particularly CIN III were DRB1*0401-DQB1*0301 and DRB1*1101-DQB1*0301. Two other DR4 associated haplotypes DRB1*0401-DQB1*0302 and DRB1*0403-DQB1*0302 also had a significant correlation with CIN. The major susceptibility haplotypes are different to those reported by Apple et al³⁰⁹ and this may be partly explained by ethnic differences as they examined a Hispanic population. Nevertheless DR4 associated haplotypes have been identified in both studies as significant although the individual alleles are different. In this study the haplotypes DRB1*0401-DQB1*0301 (n=22), DRB1*0401-DQB1*0302 (n=23) and DRB1*0404-DQB1*0302 (n=21) were the most frequent in controls, and representative of a Caucasian population²⁸⁶⁻²⁸⁷. In contrast, the haplotypes DRB1*0407-DQB1*0302 (n=18) and DRB1*0404-DQB1*0302 (n=14) were the most common in the Hispanic population³⁰⁹. It is probable that both studies together suggest that the DR4 associated haplotypes confer increased risk in the development of CIN. The other major susceptibility haplotype DRB1*1101-DQB1*0301 in this study, was not identified as significant in the Hispanic study³⁰⁹. Instead DRB1*1501-DQB1*0602 was reported as a positively associated haplotype, despite both being observed at comparable frequencies in the control population. This is likely to be a genuine difference between the studies, particularly as a protective effect was observed with DRB1*1101-DQB1*0301 in their study³⁰⁹. Two further haplotypes, less common in Caucasians, i.e. DRB1*0701-DQB1*0302 and DRB1*0801-DQB1*0301 were also found to be significant in this study though the observations were few. The haplotypes were all significant when found at the heterozygous level, but additional risk for homozygosity was not observed. On further analysis, the Hardy-Weinberg law was not maintained in the controls with an excess of

homozygotes typed for DQB1. This is probably due to false negative scoring of some controls as homozygotes on PCR-SSO. However, further analyses using the individual as the unit and combining heterozygotes and homozygotes for each allele gave similar results. The linkage disequilibrium between the individual alleles of the significant haplotypes is too strong to determine the individual allele contributing to the overall risk²⁸⁶. However, in this study positively associated haplotypes all contained DQB1*03 alleles and the simplest explanation of the data is that the relevant factor is most closely linked to the DQB1*03 locus and the resulting association with DRB1*0401 or DRB1*1101 is due to linkage disequilibrium.

The haplotype DRB1*0101-DQB1*0501 was the only one found in this study to be negatively associated with CIN (O.R. 0.48, $p=0.01$). Other individual DQB1 (0402,0603,0604) and DR (DRB1*1301, DRB1*0701, DRB5*0101) alleles that were more weakly negatively associated, did not reach statistical significance at haplotype level. The protective haplotypes identified for CIN in the Hispanic study³⁰⁹ are completely different and may be partly due to genetic differences between the populations. However, significant results for protective haplotypes have to be interpreted cautiously, because of the number of comparisons and both our observations and that found in the Hispanic study may still be due to chance. This is suggested by the absence of a significant protective effect for any of the DR or DQ alleles when the data are reanalysed excluding the positively associated DRB1*04 and DQB1*03 alleles.

All the significant haplotypes correlated with HPV positive CIN. Firm conclusions could not be drawn, with respect to type specific correlation due to insufficient number. The presence of multiple HPV types (16%) in CIN, unlike cervical cancer, also dilutes the ability to delineate the contribution of an individual HPV type in calculating risks³¹⁰.

TABLE 4.1 - CORRELATION BETWEEN INDIVIDUAL DRB1, DRB3, DRB4, DRB5 ALLELES AND CIN.

| DR | Controls | CIN I | CIN III | Total | Odds ratio (95% C.I.) | | | | Trend 'p' |
|-------|----------|-------|---------|-------|-----------------------|--------|------------------|--------|-----------|
| DRB1 | | | | | CIN | p | CIN III | p | |
| 0101 | 71 | 7 | 8 | 86 | 0.48 (0.27-0.84) | 0.01 | 0.39 (0.19-0.82) | 0.01 | 0.01 |
| 0102 | 8 | 1 | 0 | 9 | 0.29 | | | | |
| 0103 | 15 | 3 | 8 | 26 | 1.76 | | 2.00 | | |
| 0301 | 132 | 24 | 38 | 194 | 1.13 | | 1.07 | | |
| 0302 | 12 | 1 | 1 | 14 | 0.39 | | 0.30 | | |
| 0401 | 53 | 12 | 30 | 95 | 1.99 (1.30-3.04) | 0.002 | 2.25 (1.40-3.60) | 0.0012 | 0.0007 |
| 0402 | 13 | 4 | 1 | 18 | 0.91 | | 0.28 | | |
| 0403 | 6 | 3 | 6 | 15 | 3.61 (1.33-9.82) | 0.019 | 3.75(1.26-11.16) | 0.03 | 0.02 |
| 0404 | 30 | 3 | 9 | 42 | 0.94 | | 1.11 | | |
| 0405 | 1 | 0 | 0 | 1 | - | - | - | - | |
| 0406 | 0 | 1 | 5 | 6 | (3.74-∞) | 0.0007 | (4.87-∞) | 0.0004 | 0.01 |
| 0407 | 4 | 1 | 3 | 8 | 2.38 | | 2.78 | | |
| 0408 | 5 | 0 | 0 | 5 | - | | - | | |
| 0410 | 4 | 0 | 0 | 4 | - | | - | | |
| 0801 | 22 | 5 | 5 | 32 | 1.08 | | 0.83 | | |
| 0802 | 3 | 1 | 3 | 7 | 3.18 | | 3.72 | | |
| 0803 | 7 | 1 | 1 | 9 | 0.67 | | 0.52 | | |
| 08031 | 1 | 0 | 1 | 2 | 2.37 | | 3.69 | | |
| 08042 | 1 | 3 | 0 | 4 | 7.14 | | - | | |
| 1101* | 24 | 8 | 15 | 47 | 2.35 (1.32-4.20) | 0.005 | 2.39 (1.25-4.60) | 0.015 | 0.006 |
| 1102 | 1 | 0 | 0 | 1 | - | | - | | |
| 1103 | 2 | 0 | 0 | 2 | - | | - | | |
| 1104 | 13 | 2 | 2 | 17 | 0.72 | | 0.56 | | |
| 1201 | 7 | 0 | 3 | 10 | 1.01 | | 1.59 | | |
| 1202 | 1 | 0 | 1 | 2 | 2.37 | | 3.69 | | |
| 1301 | 65 | 13 | 6 | 84 | 0.67 (0.40-1.13) | | 0.32 (0.14-0.74) | 0.004 | 0.03 |
| 1302 | 28 | 5 | 7 | 40 | 1.01 | | 0.92 | | |
| 1303 | 2 | 0 | 2 | 4 | 2.37 | | 3.71 | | |
| 1304 | 2 | 0 | 2 | 4 | 2.37 | | 3.71 | | |

* CINI vs controls O.R. 2.28, p=0.06

| DRB1 | Controls | CIN I | CIN III | Total | Odds ratio (95% C.I.) | | | | Trend 'p' |
|--------------|----------|-------|---------|--------|-----------------------|------|------------------|------|--------------|
| | | | | | CIN | p | CINIII | p | |
| 1305 | 5 | 0 | 0 | 5 | - | | - | | |
| 1401 | 27 | 4 | 8 | 39 | 1.05 | | 1.09 | | |
| 1402 | 2 | 2 | 0 | 4 | 2.37 | | - | | |
| 1403 | 1 | 0 | 0 | 1 | - | | - | | |
| 1404 | 4 | 0 | 0 | 4 | - | | - | | |
| 1406 | 3 | 0 | 0 | 6 | 2.38 | | 3.72 | | |
| 1407 | 3 | 0 | 0 | 3 | - | | - | | |
| 1501 | 75 | 9 | 17 | 101 | 0.8 | | 0.82 | | |
| 1502 | 17 | 3 | 7 | 27 | 1.40 | | 1.53 | | |
| 1601 | 12 | 0 | 4 | 16 | 0.79 | | 1.23 | | |
| 1602 | 3 | 0 | 3 | 6 | 2.38 | | 3.72 | | |
| 0701/0702 | 90 | 7 | 16 | 113 | 0.58 (0.36-0.92) | 0.02 | 0.63 (0.36-1.09) | 0.1 | 0.05 |
| 0901 | 37 | 3 | 8 | 48 | 0.69 | | 0.79 | | |
| 1001 | 20 | 0 | 3 | 23 | 0.35 | | 0.55 | | |
| Total | n=832 | n=126 | n=226 | n=1184 | | | | | |
| DRB3 | | | | | | | | | |
| 0101 | 139 | 25 | 30 | 194 | 0.92 | | 0.76 | | |
| 0201 | 16 | 2 | 3 | 21 | 0.73 | | 0.69 | | |
| 0202 | 96 | 16 | 33 | 145 | 1.24 | | 1.31 | | |
| 0301 | 27 | 5 | 5 | 37 | 0.87 | | 0.67 | | |
| Null | 554 | 78 | 151 | | | | | | |
| DRB4 | | | | | | | | | |
| DR53 | 200 | 27 | 57 | 284 | 0.99 | | 1.07 | | |
| Null | 632 | 99 | 169 | | | | | | |
| DRB5 | | | | | | | | | |
| 0101 | 46 | 4 | 5 | 55 | 0.45 (0.22-0.91) | 0.03 | 0.39(0.16-0.96) | 0.04 | 0.03 |
| 0102 | 15 | 2 | 7 | 24 | 1.43 | | 1.74 | | |
| 0201/2 | 35 | 5 | 18 | 58 | 1.59 | | 1.97(1.10-3.53) | 0.03 | 0.03 |
| Null | 736 | 115 | 196 | | | | | | |
| Total | n=832 | n=126 | n=226 | n=1184 | | | | | |

TABLE 4.2 - CORRELATION BETWEEN INDIVIDUAL DQ ALLELES AND CIN

| DQ | Controls | CINI | CINIII | Total | Odds Ratio (95% C.I.) | | | | Trend 'p' |
|-------|----------|-------|--------|--------|-----------------------|--------|------------------|--------|--------------|
| | | | | | CIN | p | CINIII | p | |
| 0201 | 180 | 27 | 46 | 253 | 0.95 | | 0.93 | | |
| 0301* | 79 | 22 | 51 | 152 | 2.49 (1.77-3.52) | 0.0001 | 2.78 (1.89-4.09) | 0.0001 | 0.0001 |
| 0302 | 86 | 16 | 45 | 147 | 1.82 (1.28-2.59) | 0.001 | 2.16 (1.45-3.20) | 0.0002 | 0.0002 |
| 0303 | 20 | 7 | 5 | 32 | 1.43 | | 0.92 | | |
| 0304 | 1 | 0 | 0 | 1 | - | - | - | - | |
| 0401 | 17 | 1 | 4 | 22 | 0.69 | | 0.86 | | |
| 0402 | 42 | 7 | 2 | 51 | 0.49 (0.24-1.01) | 0.06 | 0.17 (0-0.63) | 0.004 | 0.02 |
| 0501 | 89 | 9 | 10 | 108 | 0.48 (0.29-0.79) | 0.004 | 0.39 (0.20-0.75) | 0.003 | 0.003 |
| 0502 | 31 | 3 | 8 | 42 | 0.83 | | 0.95 | | |
| 0503 | 1 | 0 | 0 | 1 | - | - | - | - | |
| 05031 | 20 | 1 | 6 | 27 | 0.82 | | 1.11 | | |
| 05032 | 11 | 2 | 3 | 16 | 1.08 | | 1.00 | | |
| 0504 | 17 | 1 | 2 | 20 | 0.41 | | 0.43 | | |
| 0601 | 69 | 12 | 17 | 98 | 0.99 | | 0.90 | | |
| 0602 | 31 | 2 | 9 | 42 | 0.83 | | 1.07 | | |
| 0603 | 49 | 6 | 4 | 59 | 0.47 (0.24-0.92) | 0.03 | 0.29 (0.11-0.78) | 0.009 | 0.02 |
| 0604 | 72 | 9 | 10 | 91 | 0.6 (0.36-1.01) | 0.06 | 0.49 (0.25-0.95) | 0.04 | 0.04 |
| 0605 | 17 | 1 | 3 | 21 | 0.55 | | 0.64 | | |
| 0606 | 0 | 0 | 1 | 1 | - | - | - | - | |
| Total | n=832 | n=126 | n=226 | n=1184 | | | | | |

* CIN I vs controls O.R. 2.02, p<0.01.

TABLE 4.3 - CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL DQB1 AND DRB1 ALLELES AND HPV

| DQB1 | Controls | HPV- CIN+ | HPV+ CIN+ | Total | Odds ratio (95% C.I.) HPV+CIN vs Controls | 'p' |
|-------------|-----------------|------------------|------------------|--------------|--|------------|
| 0301 | 79 | 14 | 59 | 152 | 2.77 (1.91-4.01) | 0.00001 |
| 0302 | 86 | 15 | 46 | 147 | 1.85 (1.25-2.72) | 0.003 |
| 0402 | 42 | 5 | 4 | 51 | 0.29 (0.11-0.79) | 0.01 |
| 0501 | 89 | 3 | 16 | 108 | 0.54 (0.31-0.94) | 0.03 |
| 0603 | 49 | 3 | 7 | 59 | 0.44 (0.20-0.96) | 0.04 |
| 0604 | 72 | 6 | 13 | 91 | 0.55 (0.30-1.00) | 0.06 |
| DRB1 | | | | | | |
| 0101 | 71 | 2 | 13 | 86 | 0.56 (0.31-1.02) | 0.06 |
| 0401 | 53 | 6 | 36 | 95 | 2.34 (1.50-3.66) | 0.0004 |
| 0403 | 6 | 3 | 6 | 15 | 3.23 (1.09-9.58) | 0.04 |
| 0406 | 0 | 0 | 6 | 6 | (5.05-∞) | 0.0002 |
| 1101 | 24 | 7 | 16 | 47 | 2.19 (1.16-4.15) | 0.02 |
| 1301 | 65 | 8 | 11 | 84 | 0.52 (0.27-0.99) | 0.05 |
| DRB5 | | | | | | |
| 0101 | 46 | 3 | 6 | 55 | 0.40 (0.17-0.93) | 0.03 |
| Total | n=832 | n=90 | n=262 | n=1184 | | |

TABLE 4.4 - CORRELATION BETWEEN DR/DQ HAPLOTYPES AND CIN

| DR/DQ haplotype* | Controls | CIN I | CIN III | Total | Odds ratio (95% C.I.) | | | | Trend 'p' |
|------------------|----------|-------|---------|--------|----------------------------|-------|----------------------------|-------|-----------|
| | | | | | CIN vs Controls. | 'p' | CIN 3 vs Controls | 'p' | |
| 0101/0501 | 66 | 7 | 7 | 80 | 0.48 (0.27-0.86) | 0.02 | 0.37 (0.17-0.81) | 0.01 | 0.01 |
| 0103/0301 | 13 | 2 | 6 | 21 | 1.47 | | 1.72 | | |
| 0301/0201 | 127 | 22 | 38 | 187 | 1.14 | | 1.12 | | |
| 0401/0301 | 22 | 5 | 15 | 42 | 2.22 (1.20-4.08) | 0.02 | 2.62 (1.35-5.08) | 0.007 | 0.005 |
| 0401/0302 | 23 | 5 | 13 | 41 | 1.90 (1.02-3.53) | 0.05 | 2.15 (1.08-4.26) | 0.04 | 0.03 |
| 0402/0302 | 10 | 2 | 1 | 13 | 0.71 | | 0.37 | | |
| 0403/0302 | 5 | 3 | 6 | 14 | 4.34 (1.51-12.43) | 0.007 | 4.51 (1.45-14.05) | 0.02 | 0.01 |
| 0404/0302 | 21 | 3 | 6 | 30 | 1.01 | | 1.05 | | |
| 0406/0302 | 0 | 1 | 3 | 4 | ∞ (2.48- ∞) | 0.008 | ∞ (2.90- ∞) | 0.009 | 0.03 |
| 0406/0301 | 0 | 0 | 2 | 2 | ∞ (1.23- ∞) | 0.09 | ∞ (1.93- ∞) | 0.05 | |
| 0701/0201 | 36 | 3 | 5 | 44 | 0.51 (0.24-1.10) | 0.09 | 0.50 (0.20-1.25) | 0.18 | |
| 0701/0302† | 8 | 0 | 8 | 16 | 2.40 (0.92-6.21) | 0.1 | 3.78 (1.45-9.84) | 0.009 | 0.01 |
| 0801/0402 | 18 | 3 | 1 | 22 | 0.52 (0.18-1.48) | 0.3 | 0.20 (0-1.19) | 0.09 | |
| 1101/0301‡ | 8 | 4 | 9 | 21 | 3.95 (1.66-9.37) | 0.003 | 4.27 (1.68-10.85) | 0.004 | 0.002 |
| 1101/0603 | 12 | 3 | 2 | 17 | 0.98 | | 0.61 | | |
| 1301/0303§ | 9 | 5 | 1 | 15 | 1.59 | | 0.41 | | |
| 1301/0603 | 19 | 3 | 1 | 23 | 0.49 (0.17-1.39) | 0.25 | 0.19 (0-1.12) | 0.1 | |
| 1301/0604 | 30 | 3 | 3 | 36 | 0.46 | | 0.36 | | |
| 1302/0604 | 23 | 5 | 5 | 33 | 1.03 | | 0.80 | | |
| 1401/05031 | 14 | 1 | 4 | 19 | 0.84 | | 1.05 | | |
| 1501/0601 | 42 | 7 | 10 | 59 | 0.95 | | 0.87 | | |
| 1501/0602 | 18 | 0 | 6 | 24 | 0.78 | | 1.23 | | |
| 1502/0601 | 13 | 2 | 5 | 20 | 1.28 | | 1.43 | | |
| 1601/0502 | 11 | 0 | 4 | 15 | 0.86 | | 1.34 | | |
| Total | n=832 | n=126 | n=226 | n=1184 | | | | | |

* DR/DQ haplotypes where there were 10 or more total alleles or for which a †significant association was found.

‡ CIN I vs controls O.R. 3.38, p=0.06; § CIN I vs controls O.R. 3.78, p=0.03.

TABLE 4.5 - CORRELATION BETWEEN SIGNIFICANT DR/DQ HAPLOTYPES AND HPV

| DR/DQ haplotype | Controls | HPV-CIN+ | HPV+CIN+ | Total | Odds ratio (95% C.I.) HPV+CIN vs Controls | 'p' |
|-----------------|----------|----------|----------|--------|---|-------|
| 0101/0501 | 66 | 2 | 12 | 80 | 0.56 (0.30-1.04) | 0.07 |
| 0401/0301 | 22 | 1 | 19 | 42 | 2.88 (1.55-5.36) | 0.001 |
| 0401/0302 | 23 | 5 | 13 | 41 | 1.84 (0.93-3.64) | 0.1 |
| 0403/0302 | 5 | 3 | 6 | 14 | 3.88 (1.25-12.06) | 0.03 |
| 0406/0302 | 0 | 0 | 4 | 4 | (3.34- ∞) | 0.003 |
| 0406/0301 | 0 | 0 | 2 | 2 | (1.66- ∞) | 0.06 |
| 0701/0302 | 8 | 0 | 8 | 16 | 3.24 (1.25-8.43) | 0.03 |
| 0801/0301 | 1 | 0 | 3 | 4 | 9.63 (1.37- ∞) | 0.05 |
| 1101/0301 | 8 | 3 | 10 | 21 | 4.09 (1.64-10.16) | 0.004 |
| Total | n=832 | n=90 | n=262 | n=1184 | | |

TABLE 4.6 - CORRELATION BETWEEN SIGNIFICANT DRB1/DQB1 HAPLOTYPES AND HPV TYPE

| | | DRB1/DQB1 haplotypes | | | | | | | | | | | | | | |
|----------------------|-----|----------------------|----------------|-------|-----------|------|------|-----------|---------------|-------|-----------|-------------------|------|-----------|------|-----|
| | | 0401/0301 | | | 0401/0302 | | | 1101/0301 | | | 0701/0302 | | | 0101/0501 | | |
| HPV type | No | N | O. R. (95% CI) | 'p' | N | O.R. | 'p' | N | O.R. (95% CI) | 'p' | N | O.R. | 'p' | N | O.R. | 'p' |
| 16 | 150 | 10 | 2.63 (1.2-5.6) | 0.02 | 8 | 1.98 | 0.09 | 8 | 5.80 (2.2-15) | 0.001 | 5 | 3.55 (1.22-10.05) | 0.04 | 8 | 0.65 | 0.4 |
| 18 | 18 | 1 | 2.17 | 0.4 | 1 | 2.07 | 0.4 | 0 | 0 | - | 0 | 0 | - | 1 | 0.7 | 1 |
| 31 | 34 | 3 | 3.56 | 0.07 | 1 | 1.07 | 0.6 | 2 | 6.44 (1.3-32) | 0.05 | 0 | 0 | - | 0 | 0 | 0.1 |
| 33 | 18 | 0 | 0 | - | 1 | 2.07 | 0.4 | 0 | 0 | - | 1 | 6.06 | 0.2 | 1 | 0.7 | 1 |
| Multiple | 42 | 5 | 4.98 (1.9-13) | 0.007 | 2 | 1.76 | 0.3 | 0 | 0 | - | 2 | 5.15 | 0.08 | 2 | 0.58 | 0.8 |
| Controls (HPV-ve) | 832 | 22 | 1* | | 23 | 1* | | 8 | 1* | | 8 | 1* | | 66 | 1* | |

* reference category

O.R. odds ratio, (95% CI)

CHAPTER 5: POOL SEQUENCING OF NATURALLY PROCESSED PEPTIDES BOUND TO HLA-DQB1*0301 AND DQB1*0501; PREDICTION OF PEPTIDE MOTIFS FROM HUMAN PAPILLOMAVIRUS TYPE 16

- 5.1 INTRODUCTION AND PRINCIPLES**
- 5.2 ELUTED POOL SEQUENCE DATA FROM PEPTIDES ELUTED FROM HLA DQB1*0501 AND DQB1*0301**
- 5.3 PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM L1, L2, E6 AND E7 OF HPV 16 TO HLA DQB1*0301 AND DQB1*0501**
- 5.4 PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM L1, L2, E6 AND E7 OF HPV 16 TO HLA DRB1*0101 AND 0401**
- 5.5 SUMMARY AND DISCUSSION**

5.1 INTRODUCTION

The pioneering work of Buus et al³¹¹ was the first that detailed the acid extraction of naturally processed self peptides bound to MHC molecules. The application and refinement of this technique has produced substantial information on HLA-associated peptides. Thus, it has been shown that the majority of peptides that associate with HLA class I are 8-10 residues long, with allotype specific binding motifs containing up to three anchor positions^{169,312}. This is consistent with the multiple pockets and the close-ended structure of the HLA class I peptide binding groove. However, only a few peptide side-chains are actively involved, as the majority of the binding energy is obtained through conserved binding sites at the terminal ends of the peptide and extensive hydrogen-bonding networks along the peptide backbone³¹³⁻³¹⁴.

To further the understanding of the mechanisms of HLA associated susceptibility to HPV induced cervical carcinogenesis, it is reasonable to suppose that susceptibility to HPV infection reflects the presence or absence of immunodominant peptide binding motifs. Therefore, the identification of the type of peptides bound by the susceptibility and protective HLA molecules may contribute to the understanding of HPV induced cervical carcinogenesis. Furthermore, knowledge of the motif requirements of peptide binding to these molecules may allow the modification of the immune response to the human papillomavirus.

In this study, the haplotype HLADRB1*0401-DQB1*0301 was shown to correlate with susceptibility to HPV and CIN while DRB1*0101-DQB1*0501 indicated protection. The extended 3-locus haplotypes, DQA1*0301-DQB1*0301-DRB1*0401 and DQA1*0301-DQB1*0302-DRB1*0401 have been found at a frequency of 23.1% in British caucasoids²⁸⁷. Similarly, DQA1*0101-DQB1*0501-DRB1*0101 occurs at a frequency of 19.8% in British caucasoids²⁸⁷. Therefore, in the present analysis, the peptide pools eluted

from HLA {DQA1*0301/DQB1*0301} and {DQA1*0101/DQB1*0501} were sequenced (Since these significant haplotypes cover more than 20% of the British population). Amino acid preferences based on peptide sequence alignment with HPV 16 L1, L2, E6 and E7 are discussed.

5.2 ELUTED POOL SEQUENCE DATA FROM PEPTIDES ELUTED FROM HLA DQB1*0501 AND DQB1*0301

A representative SDS-PAGE analysis of HLA DQA1*0101-DQB1*0501 from the JESTHOM cell line is shown in fig 5.1. The rpHPLC absorption profile for (210nm) for eluted peptides from HLA DQA1*0101-DQB1*0501 and HLA DQA1*0301-DQB1*0301 are shown in figures 5.2 and 5.3 respectively. The profiles illustrate the heterogeneity of the eluted protein material. Pooled fractions from the eluted HLA DQA1*0101-DQB1*0501 and HLA DQA1*0301-HLA DQB1*0301 were used for further analysis (Edman sequencing). Only 50% of the HPLC fractions were used for this pool.

Tables 5.1 and 5.2 show the pool sequencing data for HLA DQA1*0101-DQB1*0501 and HLA DQA1*0301-DQB1*0301. Norvaline (nv) was used as solvent to dissolve PTH amino acid and to verify the injection. Norleucine was used as standard. For interpretation of the data, the amount of increase of yield of PTH-amino acid at each cycle was considered more significant than the actual number itself. Two levels of arbitrary significance were employed. Values at least 50% higher as compared with either of the three previous cycles are considered highly significant (similar to the evaluation in Falk et al¹⁶⁹). Signals with high absolute values and either a small increase as compared to the previous cycle, or a decrease lower than the expected lag for the residue are considered likely to be presented in a proportion of peptides in the mixture. These significant residues are underlined. In this way, pool sequence data for DQB1*0501 and DQB1*0301 ligands were obtained.

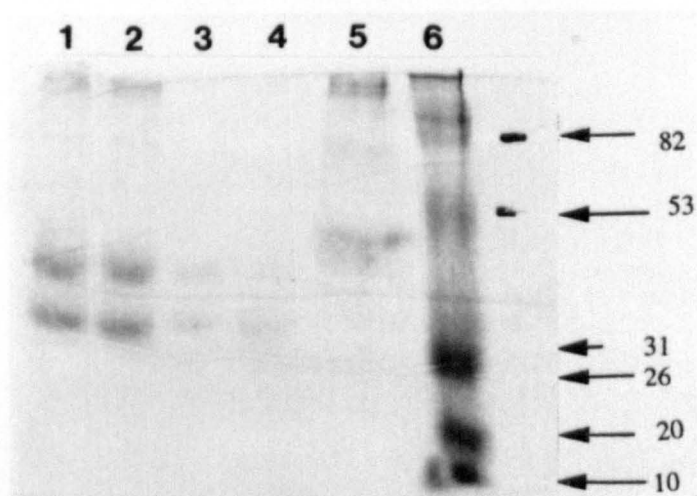


Fig 5.1: 12% SDS-PAGE Analysis of HLA DQA1*0101-DQB1*0501 obtained from the JESTHOM cell line. Lane 1: DR-1 (control); Lane 2: DQB1*0501 from JESTHOM; Lane 3: DR1; Lane 4: DQB1*0501; Lane 5: Flow through column; Lane 6: Marker.

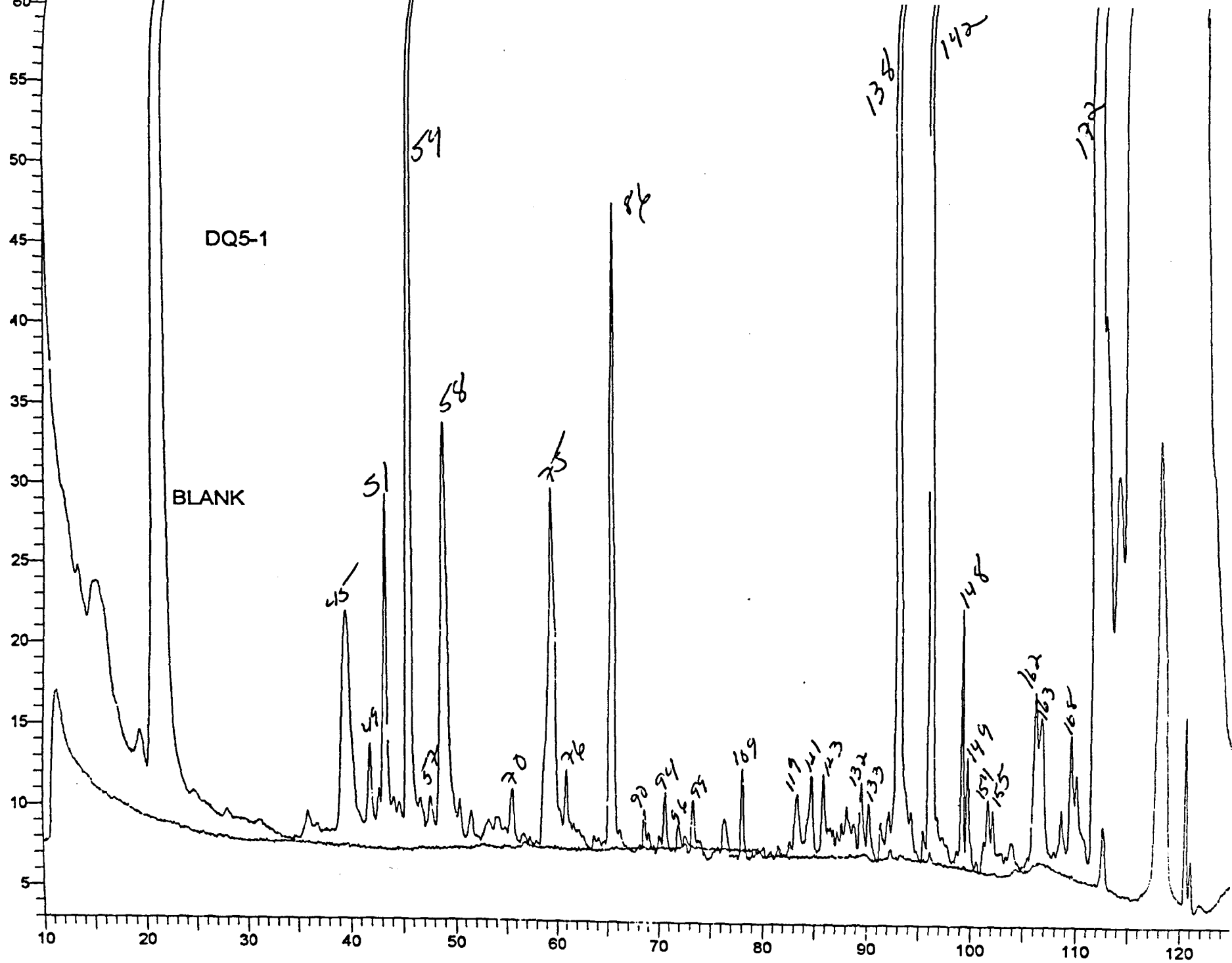


Fig 5.2: HLA DQB1*0501 peptide pools were separated by r-HPLC. Each HPLC chromatogram represents the peptide repertoire as detected by UV absorbance at 210nm

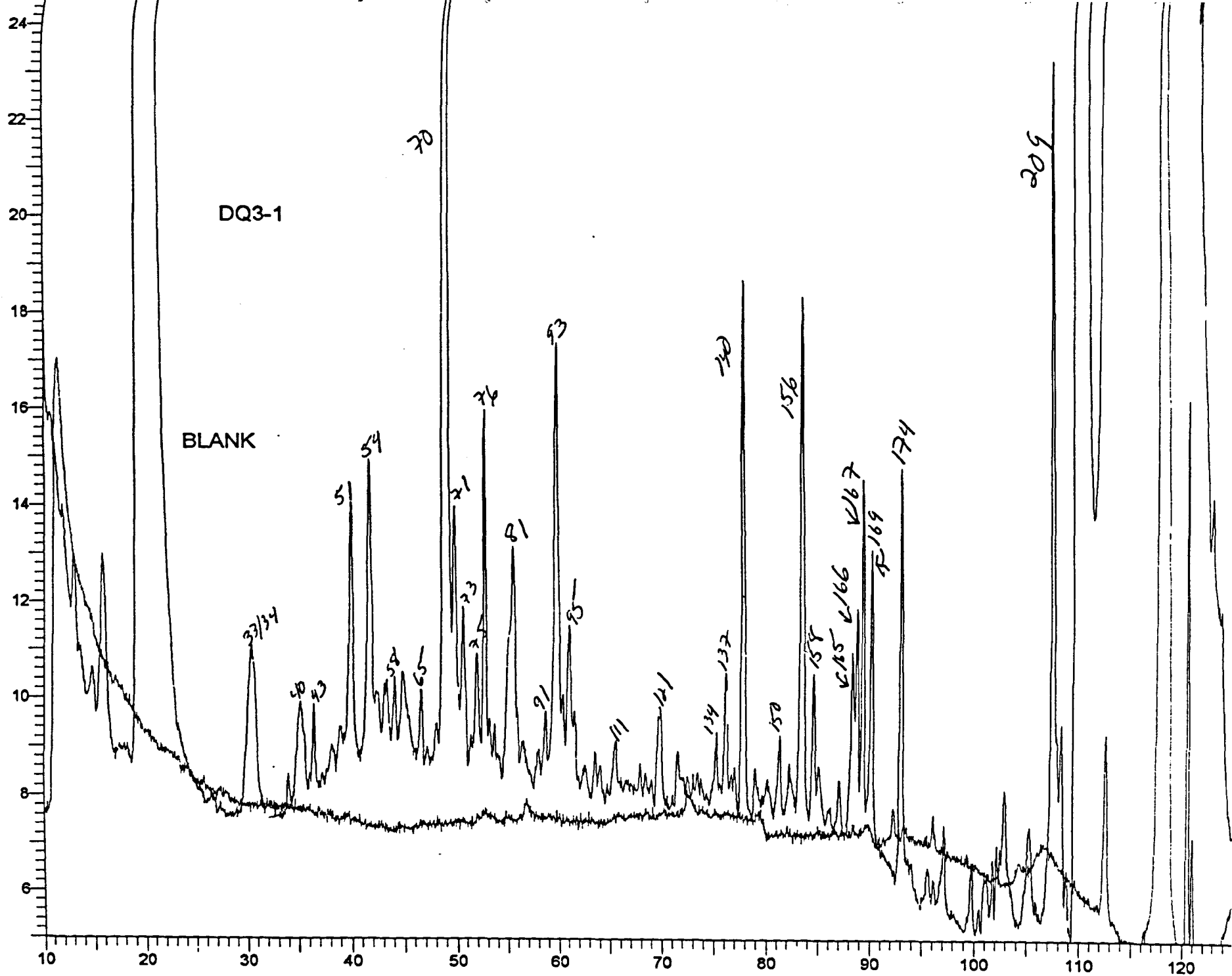


Fig 5.3: HLADQB1*0301 peptide pools were separated by r-HPLC. Each HPLC chromatogram represents the peptide repertoire as detected by UV absorbance at 210nm

| cycle | A | D | E | F | G | H | I | K | L | M | N | P | Q | R | S | smc | T | V | W | Y | nl | nv |
|-------|-------------|-------------|------|-------------|-------------|------|-------------|-------------|-------------|-------------|-------------|-------------|------|-------------|-------|------|-------------|------|------|-------------|------|------|
| 1 | 22.18 | 14.27 | 6.78 | 17.36 | 15.09 | 2.62 | 0.00 | 11.76 | 6.78 | 1.94 | 3.24 | 1.90 | 0.00 | 0.32 | 26.00 | 0.02 | 11.00 | 6.28 | 0.00 | 3.77 | 4.55 | 2.53 |
| 2 | 5.01 | 6.96 | 4.14 | 2.20 | 10.05 | 1.69 | 2.84 | 4.54 | 4.59 | 0.54 | 1.75 | <u>2.17</u> | 1.68 | 4.40 | 9.25 | 0.01 | 3.50 | 3.03 | 0.00 | 2.12 | 0.22 | 1.65 |
| 3 | 2.30 | 5.63 | 3.39 | 1.18 | 6.70 | 1.29 | 2.00 | 2.63 | 2.94 | 0.48 | 1.63 | 2.01 | 1.14 | 4.10 | 3.92 | 0.02 | 2.00 | 1.84 | 0.00 | 2.02 | 0.03 | 1.52 |
| 4 | 1.67 | 3.89 | 3.09 | 1.02 | 4.07 | 0.94 | 1.57 | 1.36 | 2.56 | 0.27 | 1.18 | 1.36 | 1.60 | 4.20 | 1.62 | 0.03 | 1.00 | 1.32 | 0.00 | 1.03 | 0.06 | 1.40 |
| 5 | 1.74 | 3.05 | 2.21 | 0.80 | 2.77 | 0.68 | 1.48 | 0.66 | 2.22 | 0.27 | <u>1.38</u> | 0.98 | 1.37 | <u>5.49</u> | 1.40 | 0.01 | <u>1.16</u> | 1.17 | 0.00 | 0.84 | 0.06 | 1.35 |
| 6 | <u>1.86</u> | 2.92 | 2.03 | 0.98 | 2.42 | 0.61 | <u>1.93</u> | 0.67 | <u>2.53</u> | <u>0.33</u> | 1.06 | <u>1.07</u> | 1.20 | 4.25 | 1.20 | 0.03 | 1.03 | 1.07 | 0.00 | 0.97 | 4.08 | 1.34 |
| 7 | 1.69 | 1.92 | 1.36 | 1.00 | 1.65 | 0.44 | 0.00 | <u>2.14</u> | 1.34 | 0.28 | 1.01 | 0.99 | 0.79 | 3.15 | 0.99 | 0.01 | 0.76 | 0.85 | 0.00 | 0.90 | 0.32 | 1.23 |
| 8 | <u>2.27</u> | 1.83 | 1.19 | 0.93 | <u>1.81</u> | 0.41 | 0.00 | 1.79 | 1.43 | 0.25 | 0.75 | 0.79 | 0.78 | <u>3.51</u> | 1.04 | 0.01 | 0.86 | 0.98 | 0.00 | 0.84 | 0.04 | 1.30 |
| 9 | 1.36 | <u>2.06</u> | 1.04 | 0.61 | 1.34 | 0.37 | 0.00 | <u>2.29</u> | <u>1.86</u> | 0.20 | 0.70 | 0.73 | 0.74 | 3.10 | 0.85 | 0.02 | 0.60 | 0.82 | 0.00 | 0.52 | 0.03 | 1.29 |
| 10 | 1.17 | 1.43 | 0.80 | 0.44 | 1.02 | 0.29 | 0.00 | <u>2.43</u> | 1.03 | 0.14 | 0.57 | 0.55 | 0.80 | 2.27 | 0.62 | 0.01 | 0.41 | 0.60 | 0.00 | 0.35 | 0.05 | 1.21 |
| 11 | <u>1.55</u> | 1.21 | 0.71 | 0.38 | 1.11 | 0.23 | 0.00 | 0.00 | 0.69 | 0.10 | 0.50 | 0.49 | 0.59 | 2.08 | 0.67 | 0.01 | 0.41 | 0.52 | 0.00 | 0.32 | 2.89 | 1.21 |
| 12 | 1.29 | <u>1.19</u> | 0.88 | <u>0.47</u> | <u>1.44</u> | 0.29 | 0.00 | 1.86 | 0.78 | 0.14 | 0.50 | 0.47 | 0.66 | 2.37 | 0.77 | 0.01 | 0.46 | 0.86 | 0.00 | 0.37 | 0.49 | 1.31 |
| 13 | 0.81 | 0.89 | 0.84 | 0.28 | 1.05 | 0.21 | 0.00 | 1.66 | 0.79 | 0.12 | 0.45 | 0.37 | 0.55 | 2.37 | 0.57 | 0.01 | 0.37 | 0.53 | 0.00 | 0.32 | 0.20 | 1.23 |
| 14 | 0.65 | 0.84 | 0.58 | 0.25 | 0.78 | 0.16 | <u>0.54</u> | 0.06 | 0.50 | 0.07 | 0.43 | 0.33 | 0.42 | <u>3.15</u> | 0.42 | 0.01 | 0.24 | 0.45 | 0.00 | 0.25 | 0.37 | 1.19 |
| 15 | 0.64 | 0.70 | 0.47 | 0.27 | 0.77 | 0.20 | 0.00 | 1.05 | 0.58 | 0.04 | 0.36 | 0.33 | 0.43 | <u>3.36</u> | 0.43 | 0.01 | 0.32 | 0.56 | 0.00 | <u>0.42</u> | 0.00 | 1.25 |
| 16 | 0.69 | 0.62 | 0.41 | 0.26 | 0.78 | 0.17 | 0.00 | 1.06 | 0.46 | 0.11 | 0.28 | 0.32 | 0.44 | 2.42 | 0.41 | 0.01 | 0.26 | 0.41 | 0.00 | <u>0.77</u> | 1.78 | 1.28 |
| 17 | 0.58 | 0.56 | 0.39 | <u>0.22</u> | 0.80 | 0.23 | 0.48 | 0.11 | 0.46 | 0.11 | 0.27 | 0.27 | 0.38 | 1.97 | 0.40 | 0.02 | 0.35 | 0.39 | 0.00 | 0.45 | 0.54 | 1.27 |
| 18 | 0.59 | 0.46 | 0.33 | 0.25 | 0.73 | 0.16 | 0.00 | 0.96 | 0.45 | 0.09 | 0.27 | 0.25 | 0.36 | 1.67 | 0.38 | 0.01 | 0.53 | 0.36 | 0.00 | 0.30 | 0.00 | 1.27 |
| 19 | 0.54 | 0.47 | 0.28 | 0.22 | 0.62 | 0.12 | 0.56 | 0.05 | 0.41 | 0.07 | 0.25 | 0.23 | 0.33 | 1.50 | 0.35 | 0.01 | 0.14 | 0.35 | 0.00 | 0.43 | 0.00 | 1.26 |

Table 5.1: Sequencing of DQB1*0501 ligands. The pools were sequenced by Edman degradation. The numbers indicate pmols of individual amino acids residues detected at each cycle. SMC = S-methyl cysteine; nl = norleucine (standard); nv = norvaline.

Amino acid residues (in pmol)

| cycle | A | D | E | F | G | H | I | K | L | M | N | P | Q | R | S | smc | T | V | W | Y | nl | nv |
|-------|-------------|-------|-------------|-------------|-------------|------|-------------|-------|------|------|------|-------------|-------------|-------------|-------|------|-------------|-------------|------|-------------|------|------|
| 1 | 24.48 | 14.63 | 8.55 | 6.78 | 18.71 | 2.97 | 8.92 | 18.00 | 6.40 | 1.55 | 4.59 | 2.25 | 3.93 | 10.77 | 30.89 | 0.37 | 14.10 | 7.02 | 0.00 | 3.93 | 8.47 | 3.39 |
| 2 | 6.23 | 7.93 | 4.75 | 2.56 | 10.39 | 2.48 | 4.03 | 6.99 | 2.82 | 0.81 | 2.89 | 6.31 | 2.41 | 5.60 | 10.90 | 0.17 | 4.39 | 3.34 | 0.00 | 1.76 | 0.08 | 1.79 |
| 3 | 5.32 | 6.78 | 4.46 | 1.91 | 6.42 | 1.93 | 2.02 | 4.91 | 1.85 | 1.09 | 0.00 | 2.82 | 1.65 | 4.32 | 4.95 | 0.07 | 1.76 | 1.99 | 0.00 | 1.22 | 0.00 | 1.52 |
| 4 | 5.70 | 7.36 | <u>5.44</u> | 0.67 | 4.86 | 1.40 | 1.06 | 3.51 | 1.39 | 0.92 | 0.00 | 1.70 | 1.61 | 4.62 | 2.06 | 0.05 | 1.40 | 1.59 | 0.00 | 0.84 | 0.04 | 1.38 |
| 5 | 5.68 | 4.04 | 4.88 | 0.87 | 4.13 | 1.28 | 0.77 | 3.26 | 1.17 | 0.55 | 0.00 | 1.33 | 1.35 | <u>5.40</u> | 1.63 | 0.08 | <u>2.51</u> | 1.42 | 0.00 | 0.73 | 0.04 | 1.35 |
| 6 | <u>8.06</u> | 2.18 | 3.94 | 0.55 | 3.79 | 0.84 | 0.70 | 2.01 | 0.98 | 0.44 | 0.97 | 1.37 | 1.46 | 4.62 | 1.45 | 0.04 | 1.02 | 1.86 | 0.00 | 0.55 | 6.41 | 1.34 |
| 7 | <u>8.47</u> | 1.38 | 2.17 | <u>0.82</u> | <u>4.20</u> | 0.69 | <u>0.95</u> | 1.88 | 0.97 | 0.30 | 0.80 | 1.09 | <u>1.47</u> | 4.19 | 1.43 | 0.03 | 0.84 | <u>1.89</u> | 0.00 | <u>1.57</u> | 0.26 | 1.37 |
| 8 | 7.43 | 1.00 | 1.25 | 0.46 | 3.23 | 0.00 | 0.49 | 1.27 | 1.13 | 0.26 | 0.79 | <u>1.37</u> | 1.43 | 4.52 | 1.30 | 0.02 | 0.93 | 1.79 | 0.00 | 0.52 | 0.05 | 1.31 |
| 9 | 7.46 | 0.82 | 0.93 | 0.30 | 1.78 | 0.00 | <u>1.00</u> | 1.38 | 1.14 | 0.20 | 0.78 | <u>1.50</u> | 1.02 | <u>4.66</u> | 1.55 | 0.02 | 0.72 | <u>2.52</u> | 0.00 | 0.40 | 0.00 | 1.30 |
| 10 | 5.65 | 0.70 | 0.87 | 0.29 | 1.70 | 0.00 | 0.54 | 0.99 | 0.89 | 0.24 | 0.77 | 1.48 | 0.99 | 4.15 | 1.22 | 0.05 | <u>1.27</u> | <u>2.76</u> | 0.00 | 0.28 | 0.05 | 1.30 |
| 11 | 5.14 | 0.58 | 0.70 | 0.24 | 1.83 | 0.50 | 0.95 | 0.23 | 0.55 | 0.23 | 0.67 | 1.45 | 0.90 | 3.15 | 1.04 | 0.02 | 0.61 | 1.47 | 0.00 | 0.26 | 5.38 | 1.30 |
| 12 | 3.31 | 0.27 | 0.59 | 0.15 | 1.67 | 0.00 | 0.47 | 0.27 | 0.59 | 0.18 | 0.33 | 1.54 | 0.81 | 3.01 | 0.98 | 0.01 | 0.44 | 1.01 | 0.00 | 0.23 | 0.34 | 1.25 |
| 13 | 2.41 | 0.61 | 0.61 | 0.17 | 1.77 | 0.00 | 0.29 | 0.42 | 0.59 | 0.13 | 0.56 | 0.93 | 0.79 | 2.47 | 0.70 | 0.01 | 0.45 | 0.63 | 0.00 | 0.16 | 0.08 | 1.29 |
| 14 | 1.40 | 0.54 | 0.52 | 0.19 | 1.16 | 0.79 | 0.26 | 0.46 | 0.51 | 0.06 | 0.49 | 0.95 | 0.52 | 2.11 | 0.66 | 0.01 | 0.23 | 0.42 | 0.00 | 0.13 | 0.05 | 1.29 |
| 15 | 0.95 | 0.21 | 0.39 | 0.17 | 0.88 | 0.00 | 0.00 | 0.00 | 0.52 | 0.08 | 0.27 | 0.68 | 0.58 | 1.54 | 0.58 | 0.03 | 0.27 | 0.36 | 0.00 | 0.10 | 0.04 | 1.28 |
| 16 | 0.65 | 0.27 | 0.32 | 0.15 | 0.83 | 0.00 | 0.12 | 0.00 | 0.30 | 0.07 | 0.41 | 0.51 | 0.47 | 1.27 | 0.47 | 0.01 | 0.25 | 0.26 | 0.00 | 0.12 | 2.42 | 1.26 |
| 17 | 0.50 | 0.20 | 0.27 | 0.08 | 0.68 | 0.00 | 0.00 | 0.00 | 0.29 | 0.15 | 0.29 | 0.40 | 0.42 | 0.93 | 0.37 | 0.01 | 0.22 | 0.24 | 0.00 | 0.13 | 0.21 | 1.30 |

Table 5.2: Sequencing of DQB1*0301 ligands. The pools were sequenced by Edman degradation. The numbers indicate pmols of individual amino acids residues detected at each cycle. SMC = S-methyl cysteine; nl = norleucine (standard); nv = norvaline.

Based on the above pool sequence data, the peptide ligand for HLA DQA1*0101/DQB1*0501 is as follows:

| | | | | | | | | | | | |
|-----------|---|--------|----|--------|--------|--------|-------|-------|-------|-------|--|
| | | | | | (Leu) | | (Leu) | (Val) | (Thr) | (Ile) | |
| | | | | (Asn) | (Ile) | | (Gly) | (Asp) | | | |
| Sequence: | X | -(Pro) | -X | -(Gln) | -(Arg) | -(Phe) | -X | -Ala | -Leu | -X | |
| CYCLE # : | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |

| | | | | | | | |
|-----------|-----|-------|----|------|------|------|-------|
| | | (Glu) | | | Val | | (Thr) |
| | | (Gly) | | | | | (His) |
| Sequence: | Ala | -Val | -X | -Arg | -Tyr | -Tyr | -Thr |
| CYCLE# | 11 | 12 | 13 | 14 | 15 | 16 | 17 |

This sequence shows that proline gives an outstanding signal at position 2. This most likely reflects the consequence of processing and not of MHC-binding requirements. In support of this notion is the absence of the influence of Pro residues in peptide binding studies^{268,315}. This Pro residue might protect the epitope from degradation by exopeptidases some of which, like aminopeptidase N, are hindered by prolines.

Based on the crystal structure of HLA-DR1, the peptide binding groove usually anchors a stretch of peptides which in most cases is nine amino acids long (P1 to P9). The number of amino acids between the amino terminus and P1 differs considerably between different ligands (0-10). Sequencing studies of pools of class II ligands that have had those peptides with a high copy number removed have indicated that the distance from the amino terminus to P1 is 3+/- 1 amino acids for the majority of peptides²⁶⁷. There have been only one previously reported motif for HLA-DQ1 (DQA1*0101/DQB1*0501)²⁹³ and one for HLA DQ7 (DQA1*0501/DQB1*0301)²⁶⁷. The latter report suggested that proline gives an

outstanding signal at position 2. The DQ7 motif was shown to have three anchors focused at positions 5, 9, and 11. The first and the last are dominated by aromatic residues, whereas the middle one is mainly aliphatic and only four intermittent polar clusters were found.

The DQB1*0501 motif is characterized by Asn/Arg (amidic amino acid and amino acid with basic or positively charged side chain) at P1 (absolute position 5), suggesting that a small or polar amino acid is preferred at this position. The central amino-acids are mainly aliphatic/aromatic. P5 (absolute position 9) is the focus of a hydrophobic cluster with a small contribution by small polar residues. There is another cluster of aromatic residues towards the C- terminus.

Based on the above pool sequence data, the peptide ligand for HLA-DQA1*0301/DQB1*0301 is as follows:

| | | | | | | | | |
|-----------|-----|-------|-------|-------|-------|-------|-------|-------|
| | | | (Gly) | | (Gln) | (Gly) | (Leu) | (Ser) |
| | | | (Ser) | (Arg) | (Val) | Ala | (Pro) | (Pro) |
| Sequence: | X - | Pro - | X - | Glu - | Thr - | Ala - | Tyr - | X - |
| CYCLE# : | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | | | | | | | | X |
| | | | | | | | | (Val) |

| | | | | | |
|-----------|-------|-------|-------|-----|-------|
| | (Val) | (Ile) | (Pro) | | |
| Sequence: | Thr | - X | - X | - X | - HIS |
| CYCLE# | 10 | 11 | 12 | 13 | 14 |

This sequence also shows that proline gives an outstanding signal at position 2. This is similar to the sequence of HLA DQB1*0501 and most likely reflects the consequence of processing and not of MHC-binding requirements.

In this study, the pool sequencing data beyond Pro at position 2 shows that the DQB1*0301 motif is characterized by Thr/Arg (small polar amino acid and amino acid with positively charged side chain) at P1 (absolute position 5), suggesting that a small or polar amino acid is preferred at this position. The central amino-acids are mainly aliphatic/aromatic. P5 (absolute position 9) is the focus of an aliphatic cluster with a small contribution by small polar residues. Proline gives a signal again at absolute position 12 which is towards the C-terminus. This suggests trimming of the peptide also by carboxypeptidases which are also hindered by proline.

5.3 PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM E6, E7, L1 AND L2 of HPV 16 to HLA DQB1*0301, AND DQB1*0501

On the basis of the peptide sequence data above, the E6, E7, L1 and L2 proteins of HPV 16 were examined to identify sequences which are likely to bind to HLA DQB1*0301 and DQB1*0501. The examination was performed systematically in an overlapping fashion for 13-mer peptides.

The motif analysis of the HPV 16- E7 protein (98 amino acids) shows that a total of 26 peptides possess at least one preferred residue at P1 or P5 for binding to HLA DQB1*0301. However, since most HLA molecules require two to three anchors in a peptide for optimal binding, further analysis revealed that two of these peptides (7.7%) possess the preferred amino acid residues at both P1 and P5. These are **E768-80: CVQSTHVDIRTLE**; and **E782-94: LLMGTLGIVCPIC** (Table 5.3). By contrast, a total of 28 peptides derived from HPV 16-E7 possess at least one preferred residue at P1 or P5 for binding to HLA DQB1*0501 and one of these (3.6%), **E773-85: HVDIRTLEDLLMG**, possess the preferred residues at P1 and P5 (Table 5.4).

In the analysis of HPV 16-E₆ (158 amino acids), a total of 43 peptides possess at least one preferred residue at P1 or P5 for binding to HLA DQB1*0301. Of these, four peptides (9.3%) possess the preferred residues at both P1 and P5. These are **E₆25-37: ELQTTIHDIILEC;** **E₆51-63:DFAERDLCIVYRD;** **E₆58-70: CIVYRDGNPYAVC;** and **E₆127-140: DKKQRFHNIRGRW** (Table 5.5). In the case of HLA DQB1*0501, a total of 46 peptides from HPV 16 E₆ are probably capable of binding and four of these (8.7%) possess the preferred residues at P1 and P5 (Table 5.6).

In the analysis of the L1 protein of HPV 16 (531 amino acids), a total of 166 peptides possess at least one preferred residue at P1 or P5 for binding to HLA DQB1*0301. Of these, 14 peptides (8.4%) possess the preferred residues at both P1 and P5 (Table 5.7). The comparable predicted motifs for HLA DQB1*0501 show that a total of 128 peptides that are probably capable of binding to HPV 16 L1 derived peptides with 11 peptides (8.6%) possessing the preferred residues at P1 and P5. These peptides are listed in table 5.8.

Motif analysis of the HPV 16 L2 protein (473 amino acids) for binding to HLA DQB1*0301 revealed that a total of 216 peptides possess at least one preferred residue at P1 or P5. Of these, 22 peptides (10.2%) possess the preferred residues at both P1 and P4. These peptides are listed in table 5.9. Comparative analysis of binding to HLA DQB1*0501 reveal a total of 119 peptides with at least one preferred residue, and 7 of these (6.0%) possess the preferred residues at P1 and P5 (Table 5.10).

The finding that there are no significant differences in the number of HPV 16 derived peptides that are probably capable of binding the HLA DQB1*0501 molecule compared with HLA DQB1*0301 supports the notion that the protective effect of the former on HPV associated disease relates more to peptide binding affinity. In this way, the HLA

DQB1*0501-HPV peptide complex may lead to the generation of a more effective immune response.

Table 5.3: The predicted peptide motifs from HPV 16-E7 to HLADQB1*0301.

| HPV 16 E7 | Peptide Sequence (DQB1*0301) |
|-----------|------------------------------|
| 68-80 | CVQSTHVDIRTLE |
| 82-94 | LLMGTLGIVCPIC |

Table 5.4: The predicted peptide motifs from HPV 16-E7 to HLADQB1*0501.

| HPV 16 E7 | Peptide Sequence (DQB1*0501) |
|-----------|------------------------------|
| 73-85 | HVDIRTLEDLLMG |

Table 5.5: The predicted peptide motifs from HPV 16-E6 to HLADQB1*0301.

| HPV 16 E6 | Peptide Sequence (DQB1*0301) |
|-----------|------------------------------|
| 25-37 | ELQTTIHDIILEC |
| 51-63 | DFAERDLCIVYRD |
| 58-70 | CIVYRDGNPYAVC |
| 127-139 | DKKQRFHNIRGRW |

Table 5.6: The predicted peptide motifs from HPV 16-E6 to HLADQB1*0501.

| HPV 16 E6 | Peptide Sequence (DQB1*0501) |
|-----------|------------------------------|
| 11-23 | DPQERPRKLPQLC |
| 43-55 | QLLRREVYDFAFR |
| 51-63 | DFAFRDLCIVYRD |
| 127-139 | DKKQRFHNIRGRW |

Table 5.7: The predicted peptide motifs from HPV 16-L1 to HLADQB1*0301.

| HPV 16 L1 | Peptide Sequence (DQB1*0301) |
|-----------|------------------------------|
| 32-44 | PSEATVYLPPVPV |
| 46-58 | KVVSTDEYVARTN |
| 63-75 | AGTSRLLAVGHPY |
| 93-105 | GLQYRVFRIHLPD |
| 117-129 | YNPDTQRLVWACV |
| 151-163 | VGISGHPLLNKLD |
| 166-178 | GVDNRECISMDYK |
| 198-210 | GSPCTNVAVNPGD |
| 285-297 | HLFNRAGTVGNV |
| 316-328 | NYFPTPSGSMVTS |
| 439-451 | TLEDTYRFVTQAI |
| 453-465 | CQKHTPPAPKEDD |
| 467-479 | LKKYTFWEVNLKE |
| 507-519 | TLGKRKATPTTSS |

Table 5.8: The predicted peptide motifs from HPV 16-L1 to HLADQB1*0501

| HPV 16 L1 | Peptide Sequence (DQB1*0501) |
|------------------|-------------------------------------|
| 15-27 | ENDVNVYHIFQFM |
| 78-90 | LKKPNNNKILVPK |
| 79-91 | KKPNNNKILVPKV |
| 93-105 | GLQYRVFRIHLPD |
| 131-143 | VEVGRGQPLGVGI |
| 146-158 | HPLLNLDDTENA |
| 160-172 | AYAANAGVDNREC |
| 165-177 | AGVDNRECISMDY |
| 292-304 | TVGENVPDDLVIK |
| 417-429 | ITLTADVMTYIHS |
| 488-500 | FPLGRKFLLQAGL |

Table 5.9: The predicted peptide motifs from HPV 16-L2 to HLA DQB1*0301.

| HPV 16 L2 | Peptide Sequence (DQB1*0301) |
|-----------|------------------------------|
| 6-18 | SAKRTKRASATQL |
| 65-77 | GTGGRTGYIPLGT |
| 66-78 | TGGRTGYIPLGTR |
| 81-93 | TATDTLAPVRPPL |
| 90-102 | RPPLTVDPVGPS |
| 117-129 | AGAPYSVPSIPPD |
| 135-147 | ITTSTDTPAILD |
| 137-149 | TSTDTPAILDIN |
| 178-190 | GGHFTLSSSTIST |
| 196-208 | IPMDTFIVSTNPN |
| 205-217 | TNPNTVTSSTPIP |
| 227-239 | PNTVTSSTPIPGS |
| 247-259 | TKLITYDNPAYEG |
| 261-273 | DVDNTLYFSSNDN |
| 293-305 | ALTSRRTGIRYSR |
| 298-310 | RTGIRYSRIGNKQ |
| 339-351 | IELQTITPSTYTT |
| 373-385 | FITDTSTTPVPSV |
| 375-387 | TDTSTTPVPSVPS |
| 376-388 | DTSTTPVPSVPST |
| 417-429 | PINITDQAPSLIP |
| 455-467 | LRKRRKRLPYFFS |

Table 5.10: The predicted peptide motifs from HPV 16-L2 to HLADQB1*0501

| HPV 16 L2 | Peptide Sequence (DQB1*0501) |
|-----------|------------------------------|
| 188-200 | ISTHNYEEIPMDT |
| 267-279 | YFSSNDNSINIAP |
| 269-281 | SSNDNSINIAPDP |
| 272-284 | DNSINIAPDPDFL |
| 298-310 | RTGIRYSRIGNKQ |
| 359-371 | TSINNGLYDIYAD |
| 454-466 | MLRKRRKRLPYFF |

5.4 PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM L1, L2, E6 and E7 of HPV 16 to HLA DRB1*0101 AND DRB1*0401

The requirements for peptide binding to several HLA DR alleles have been defined by the analysis of peptide analogs and have been shown to be remarkably simple^{168,170,268 315-318}. Other than the peptide backbone, only a single hydrophobic amino acid side chain appears to be critical. This was initially demonstrated in an analysis of monosubstituted analogues of an influenza hemagglutinin peptide to bind DRB1*0101^{168,317}. Of the twelve residues examined, only substitutions for a tyrosine near the amino terminus dramatically reduced binding. The importance of this single side chain was established unequivocally by demonstrating that a peptide of equal length as the natural hemagglutinin sequence, but with all amino acids other than the tyrosine and a single lysine replaced with alanine, bound both DRB1*0101 and DRB1*0401 better than the natural peptide. The ability of simplified analogues to bind as well, if not better than, the parent T cell determinants suggest that the other amino acids in the peptide ligand either made minor contributions, were neutral, or were deleterious to binding.

More extensive quantitative studies using simplified polyalanine peptides have demonstrated for binding to both DRB1*0101 and DRB1*0401 that:

- i The optimal position for the hydrophobic amino acid in the context of a thirteen amino-acid peptide was the third position (P3).
- ii The structural requirements at P3 were quite tolerant, with aromatic being superior to aliphatic side chains for binding to DRB1*0101 and DRB1*0401
- iii An analog with tyrosine at P3 bound with an IC₅₀ value three orders of magnitude lower than a peptide with alanine at this position, and more than five orders of magnitude lower than peptides containing polar amino acids at this position³¹⁷.

The minimal requirements for binding shared by eight DR alleles suggested that the important hydrophobic side chain interacted with a subsite composed principally of conserved residues³¹⁸. An appropriate site was the relatively deep hydrophobic pocket in the region of residues 24 α , 26 α , 54 α of the α -chain and 86 β of the β -chain. The only polymorphic amino acid present in this subsite was 86 β which is either a glycine or valine in the DR proteins. The side chains in the peptides that appeared to be responsible for allele specificity were determined by correlating their common structural features with complementary polymorphic residues in the binding site. The importance of the peptide side chains was tested by incorporating them into a polyalanine backbone and was confirmed by the ability of these residues to transfer allele specificity to these simplified analogues. Although polymorphic contacts affect peptide affinity, the majority of the free energy of binding in all cases arose from interactions with the peptide backbone and the single hydrophobic amino acid at the third position. These constraints appear to orient all peptides in a similar location, forcing them to adopt a closely related conformation in the binding site. The corresponding side chain in each peptide contacted the same pocket in the binding site, regardless of the allele. This apparent similarity allows for the analysis of any DR allele by extrapolation from the DRB1*0101 crystal structure.

The data from the analysis of monosubstituted polyalanine peptides binding to a set of DR alleles indicated that the free energy of binding can be viewed as a simple sum of the interactions of the peptide side chains and backbone with distinct regions of the binding site³¹⁸⁻³¹⁹. Interactions of more than a single peptide side chain with a particular subsite of the binding cleft is unlikely because of the extended conformation the peptide adopts in the binding site. Thus, the free energy of binding can be represented as a simple polynomial with separate terms for backbone interactions and the side chains. Therefore, the apparent affinity of any sequence of common length should be predictable based on the rules of binding and data on the relative effects of the natural amino acids at each position.

A suitable database for this prediction has been constructed (Rothbard, J; Stanford University, USA, personal communication) and was used in this thesis to predict HPV peptide motifs for DRB1*0101 and DRB1*0401. The program is simple, written using Microsoft Excel and is able to parse any open reading frame into 13 amino acid peptides and calculate their affinity for DRB1*0401 and DRB1*0101.

In designing the database, the contributions of the individual side chains of a peptide from influenza hemagglutinin and tetanus toxin were measured by assaying analogs of AAYAAAKAAAAAA containing the corresponding amino acid at each position. This polyalanine peptide was chosen because alanine can be viewed as having a neutral side chain due to lack of size and charge. Consequently, any substitution could be viewed as advantageous, neutral or deleterious depending on its effect as compared to the parent peptide. Assuming that the tyrosine and the peptide backbone oriented all peptides equivalently, any differences in the IC₅₀ value was due to the effect of the added amino acid. Multiplying each of the ratios for each analogue together resulted in a composite ratio, that when multiplied by the IC₅₀ value of the parent peptide (14.7nM) resulted in a predicted IC₅₀ value that was very close to experimentally determined value for both peptides. A thorough study of prediction has been done using the ratios derived from all possible monosubstituted analogues at the central eleven positions of a simplified peptide³²⁰. Prediction data from myelin basic protein, human serum albumin show that the predicted affinity in all cases, was within a factor of four of the experimentally derived value³²⁰ Rothbard J, personal communication). This degree of error is comparable to that observed for the binding assays.

The peptide motifs of HPV 16 and their IC₅₀ based on the approach described above are attached as appendix II. The motifs with IC₅₀ less than 20nM are listed as follows: Tables 5.11 and 5.12 show the motifs derived from L1; Tables 5.13 and 5.14 show the motifs

derived from L2; Tables 5.15 and 5.16 the motifs derived from E7; and Tables 5.17 and 5.18 the motifs derived from E6.

Table 5.11: The predicted binding affinity of peptides derived from HPV 16-L1 to DRB1*0101. The first nine peptides are shown.

| HPV 16 -L1 | Peptide Sequence | IC50 DRB1*0101 (nM) |
|------------|------------------|---------------------|
| 58-70 | NIYYHAGTSRLLA | 1.50 |
| 3-15 | VTFIYILVITCYE | 1.85 |
| 398-410 | LQFIFQLCKITLT | 2.85 |
| 414-426 | MTYIHSMNSTILE | 3.25 |
| 442-454 | DTYRFVTQAIACQ | 4.42 |
| 300-312 | DLYIKGSGSTANL | 5.05 |
| 317-327 | SNYFPTPSGSMVT | 6.40 |
| 59-71 | IYYHAGTSRLLAV | 7.25 |
| 159-171 | SAYAANAGVDNRE | 22.50 |

Table 5.12: The predicted binding affinity of peptides derived from HPV 16-L1 to DRB1*0401. The first five peptides are shown.

| HPV 16 -L1 | Peptide Sequence | IC50 DRB1*0401 (nM) |
|------------|------------------|---------------------|
| 442-454 | DTYRFVTQAIACQ | 1.15 |
| 492-504 | RKFLQAGLKAKP | 3.00 |
| 94-106 | LQYRVFRIHLPDP | 4.30 |
| 3-15 | VTFIYILVITCYE | 10.50 |
| 124-136 | LVWACVGVEVGRG | 21.00 |

Table 5.13: The predicted binding affinity of peptides derived from HPV 16-L2 to DRB1*0101. The first nine peptides are shown.

| HPV 16 -L2 | Peptide Sequence | IC50 DRB1*0101 (nM) |
|------------|------------------|---------------------|
| 240-252 | PAFVTTPTKLITY | 2.70 |
| 444-456 | GDFYLHPSYYMLR | 3.35 |
| 52-64 | GVFFGGLGIGTGS | 7.20 |
| 301-313 | IRYSRIGNKQTLR | 11.50 |
| 200-212 | TFIVSTNPNTVTS | 13.00 |
| 445-457 | DFYLHPSYYMLRK | 13.00 |
| 70-82 | TGYIPLGTRPPTA | 14.00 |
| 418-430 | INITDQAPSLIPI | 19.00 |
| 161-173 | PTFTDPSVLQPPT | 32.00 |

Table 5.14: The predicted binding affinity of peptides derived from HPV 16-L2 to DRB1*0401. The first four peptides are shown.

| HPV 16 -L2 | Peptide Sequence | IC50 DRB1*0401 (nM) |
|------------|------------------|---------------------|
| 46-58 | LQYGSMGVFFGGL | 9.80 |
| 266-278 | LYFSSNDNSINIA | 11.00 |
| 391-403 | SGYIPANTTIPFG | 12.00 |
| 444-456 | GDFYLHPSYYMLR | 20.20 |

Table 5.15: The predicted binding affinity of peptides derived from HPV 16-E7 to DRB1*0101. The first four peptides are shown.

| HPV 16 -E7 | Peptide Sequence | IC50 DRB1*0101 (nM) |
|------------|------------------|---------------------|
| 81-93 | DLLMGTLGIVCPI | 45.20 |
| 80-92 | EDLLMGTLGIVCP | 230.00 |
| 52-64 | YNIVTFCCCKCDST | 300.00 |
| 55-67 | VTFCCCKCDSTLRL | 1120.00 |

Table 5.16: The predicted binding affinity of peptides derived from HPV 16-E7 to DRB1*0401. The first four peptides are shown.

| HPV 16 -E7 | Peptide Sequence | IC50 DRB1*0401 (nM) |
|------------|------------------|---------------------|
| 21-33 | DLYCYEQLNDSSE | 105.00 |
| 9-21 | HEYMLDLQPETTD | 210.00 |
| 84-96 | MGTLGIVCPICSQ | 230.00 |
| 63-75 | STLRLCVQSTHVD | 452.00 |

Table 5.17: The predicted binding affinity of peptides derived from HPV 16-E6 to DRB1*0101. The first five peptides are shown.

| HPV 16 -E6 | Peptide Sequence | IC50 DRB1*0101 (nM) |
|------------|------------------|---------------------|
| 59-71 | IVYRDGNPYAVCD | 4.00 |
| 33-45 | IILECVYCKQQLL | 12.00 |
| 142-154 | RCMSCCRSSRTRR | 12.50 |
| 75-87 | KFYISKISEYRHYC | 18.20 |
| 84-96 | RHYCYSLYGTTL | 34.50 |

Table 5.18: The predicted binding affinity of peptides derived from HPV 16-E6 to DRB1*0401. The first four peptides are shown.

| HPV 16 -E6 | Peptide Sequence | IC50 DRB1*0401 (nM) |
|------------|------------------|---------------------|
| 59-71 | IVYRDGNPYAVCD | 24.00 |
| 52-64 | FAFRDLCIVYRDG | 35.00 |
| 75-87 | KFYISKISEYRHYC | 83.00 |
| 86-98 | YCYSLYGTTLEQQ | 120.00 |

5.5 SUMMARY AND DISCUSSION

In this chapter, data obtained from immunoaffinity purification and sequencing of peptides bound to the susceptibility and protective HLA DQ alleles were used to predict peptide motifs from HPV 16. There are two limitations of this data. Firstly, sequencing of the individual peptide peaks obtained after rpHPLC would have provided additional information on both sequence match and protein source of the peptides. These peptides could then be aligned with the motif derived from the pool sequence to derive anchor residues with certainty. Indeed, an attempt was made to obtain this data using electrospray ionization mass spectrometry (LC-ESI-MS/MS) but proper identification could not be made using the parameters described by Dongre et al (1997) because of insufficient samples (Data not shown). Secondly, in order to confirm the motif derived from pool sequencing, binding studies on synthetic variants of the eluted peptides could be performed. Nevertheless, the results suggest that many more peptide motifs are capable of binding to HLA DQB1*0501 than DQB1*0301. This may partly explain the mechanisms of the association of these alleles to HPV related cervical carcinogenesis (see also chapter 6).

For HLA DQ molecules, information on ligand specificity has only recently been available. Single-substitution experiments defined a simple motif for DQA1*0301/DQB1*0301 that was quite different from the motifs recognized by DR molecules²⁹⁰. Its prominent feature is the requirement of two small and/or hydrophobic residues spaced at relative positions $i+2$ and $i+4$. However, because these features can basically be found in almost every natural peptide frame, this motif is not suitable for predicting HLA-DQ ligands. Simple motifs have also been described for the autoimmune disease-linked HLA DQA1*0501/DQB1*0201^{291,296,321} and DQA1*0301/DQB1*0302^{295,322}. As for DQA1*0301/DQB1*0302, these motifs were different from the ones recognized by DR molecules. For example, no prominent position 1 anchors were found, as indicated by Ala-

substitution experiments. Both motifs consisted mainly of inhibitory residues, with the exception of a negatively charged anchor at residue at position 9.

The tendency of HLA DQ ligands to be less dependent on the interaction of peptide side chains with the class II cleft than HLA DR ligands has recently been confirmed by the determination of a quantitative matrix-based motif for DQA1*0501/DQB1*0301³²³. This motif revealed the ability of DQA1*0501/DQB1*0301 molecules to bind peptide structures without the involvement of large peptide side chains. Based on this finding, it was possible to modify DR-selected peptide repertoires such that they lose the binding capacity for HLA DR molecules and bind exclusively to the DQA1*0501/DQB1*0301, thus demonstrating, at least in part, a complementary function of HLA DR and DQ isotypes in antigen presentation³²³. These differential binding capabilities between HLA DR and HLA DQ may maximize the diversity of peptide repertoires available for T cell recognition. This may result in an additive positive or negative effect on the immune response depending on an individual's HLA class II haplotype.

Published data for the HLA DRB1*0101 and 0401 alleles were also used for motif prediction from HPV 16. Although a threshold of 20nM was arbitrarily chosen in this study to define peptides that bind with high affinity, it has been determined that an affinity threshold of 500nM determines the capacity of a peptide to elicit a CTL response in a series of HLA-A2 motif peptides evaluated in transgenic mice and in vitro recall responses in patients with acute hepatitis infection³²⁴. There is currently no available information on the affinity threshold for HLA class II. Nevertheless, in most cases, ranking of peptides according to binding affinity for a particular HLA allele seems to correlate with their immunogenic potential.

The results include information on the binding affinity of the peptides (IC_{50}) and show a number of interesting features. For the HPV 16 L1 protein, DRB1*0101 is capable of binding twice as many peptides with high affinity ($IC_{50}<20$) than DRB1*0401 (Tables 5.11 and 5.12). Even when these HLA molecules bind to the same set of peptides, the binding affinity to DRB1*0101 is several orders of magnitude higher than for DRB1*0401. For example, HPV16 L₁**58-70: NIYYHAGTSRLLA** binds DRB1*0101 with an IC_{50} of 1.5. The corresponding IC_{50} for DRB1*0401 is 105 (almost 100-fold difference).

HLA DRB1*0101 is also capable of binding twice as many L2 peptides with high affinity ($IC_{50}<20$) than DRB1*0401 (Tables 5.13 and 5.14). The peptide L₂**240-252: PAFVTTPTKLITY** that binds with the highest affinity to DRB1*0101 ($IC_{50}=2.70$), binds to DRB1*0401 with an IC_{50} of 4900, a difference of over 4,000-fold.

None of the HPV16 E7 derived peptides is capable of binding to either HLA DRB1*0101 or HLA DRB1*0401 with high affinity (i.e. $IC_{50}<20$) (Tables 5.15 and 5.16). Nevertheless, the peptide that binds best to DRB1*0101, E₇**81-93: DLLMGTLGIVCPI** ($IC_{50}=45.20$) does so at two orders of magnitude better than the peptide with the highest binding affinity to DRB1*0401 (E₇**21-33: DLYCYEQLNDSSE**; $IC_{50}=105.00$). In addition, the IC_{50} for E₇81-93 with respect to DRB1*0401 is 710, an almost 20-fold difference.

Four HPV 16 E6 derived peptides are capable of binding with high affinity ($IC_{50}<20$) to HLA DRB1*0101 compared with none for HLA DRB1*0401. Peptide E₆**59-71: IVYRDGNPYAVCD** binds to DRB1*0101 with an IC_{50} of 4.00. The corresponding IC_{50} using the same peptide for DRB1*0401 is 24.00, a six fold difference in binding affinity.

In conclusion, there are significant differences in both the number and binding affinity of HPV derived peptides to the susceptibility and protective HLA DQ and DR alleles. Since peptide binding to HLA molecules is an important step in the generation of effective immune response, these differences would likely account for the observations of HLA associations with HPV induced cervical carcinogenesis. In addition, HLA molecules influence the choice between Th1 and Th2 response from CD4+ cells. A Th1 response is required to provide an adequate response to intracellular pathogens such as viruses. If a particular HPV-derived peptide is presented in the context of a susceptibility allele, it could induce a Th2 response, and disease could progress or persist. Since CD4+ T lymphocytes play a central role in the complex immune network that leads to antigen-specific reactivity, future studies (discussed in chapter 6) should be directed at identifying HLA class II specific immunodominant epitopes from HPV that may be useful for the prevention and treatment of CIN and cervical cancer.

CHAPTER 6: DISCUSSION

- 6.1 SUMMARY**
- 6.2 DISCUSSION**
- 6.3 MECHANISMS OF HLA CLASS II ASSOCIATION WITH HPV AND CIN**
- 6.4 POLYMORPHIC STRUCTURAL FEATURES OF HLA-DQ MOLECULES
ASSOCIATED WITH SUSCEPTIBILITY OR RESISTANCE TO HPV
ASSOCIATED CIN**
- 6.5 CLINICAL IMPLICATIONS : HLA TYPING AND VACCINE
DEVELOPMENT**
- 6.6 CONCLUSIONS**
- 6.7 FUTURE STUDIES**

6.1 : SUMMARY

The primary objective of this project was to examine the association between HLA-DQ and -DR alleles, the human papillomavirus and premalignant disease of the uterine cervix. This was accomplished by HPV and HLA DNA typing. The latter consisted of three phases. The first phase involved the development of a novel polymerase chain reaction-restriction fragment length polymorphism (artificial restriction fragment length polymorphism) for HLA DQB1*03 typing³⁰⁸. The results show a significant association between CIN and DQB1*03 that is only slightly stronger for CIN III than CIN I. Homozygosity at the DQB1*03 locus, was significantly associated with CIN and was more strongly related than heterozygosity, a result not reported so far in any previous studies. A significant association with HPV positive CIN and DQB1*03 was found for all HPV types tested (16,18,31,33) and homozygosity at the DQB1*03 locus was strongly associated with HPV positive CIN.

The second phase consisted of polymerase chain reaction with sequence specific primers for HLA DQB1*03. The DQB1*0301 allele was shown to be most strongly associated with CIN and HPV, but 0302 was also positively related³⁰⁸.

The third phase of HLA DNA typing involved polymerase chain reaction followed by sequence specific oligonucleotide hybridization with digoxigenin labeled probes using the 11th Histocompatibility Protocol with some modifications. This enabled the identification of susceptibility and protective HLA DQ-DR haplotypes in relation to human papillomavirus and premalignant disease of the cervix. The haplotype HLADRB1*0401-DQB1*0301 was shown to correlate with susceptibility to HPV and CIN while DRB1*0101-DQB1*0501 indicated protection³²⁵.

In an attempt to further understand HPV antigen processing events, the final phase of the project consisted of immunoaffinity purification of the susceptibility and protective HLA DQ molecules and sequencing of the naturally processed peptide sequences bound to these HLA molecules. The data obtained was used for motif prediction of HPV 16 E6, E7, L1 and L2 sequences that are probably capable of binding to these HLA molecules. Motif prediction as well as the binding affinity of predicted peptide motifs for HLA DRB1*0401 and DRB1*0101, the DR alleles associated with susceptibility and protection respectively, was accomplished using published data on the naturally processed peptide sequences bound to these molecules. The data revealed significant differences in both the number and binding affinity of the HPV 16 derived peptides to the protective and susceptibility HLA molecules.

6.2: DISCUSSION

During the course of this project, a number of studies were published on the association between HLA class II and premalignant and malignant disease of the cervix. The purpose of this section is to discuss these studies including their merits and limitations. The results from these studies have been inconsistent, probably for the following reasons:

- i. Differences in sample size.
- ii. Difficulties in obtaining representative control groups.
- iii. Methodological differences in HLA typing (obviated by PCR-based methods in more recent studies).
- iv. Lack of information on HPV status of patients and controls in many studies.

In a report from Norway, Helland and co-workers³²⁶⁻³²⁷ using polymerase chain reaction and a DNA hybridization technique, found that 67% of 213 patients with squamous cell carcinoma of the cervix carried the DQB1 gene encoding HLA DQB1*03 compared with 51% of 118 controls (RR=2.0, $p<0.002$). However, the report provided no information

on the HPV status of the patients and controls. Another report³²⁸ on a population of 66 African American women with cervical cancer using a PCR based technique showed an increased risk with HLA DQB1*03 compared with 214 controls (RR 2.3, $p=0.004$) and the risk was highest for HLA DQB1*0303 (RR 2.7, $p=0.017$). Apple et al.³⁰⁶ examined a Hispanic population of 98 women with cervical cancer and 220 controls. Although no association between cervical carcinoma and HLA DQB1*03 alone was found, an increased risk of cervical cancer was found with the DRB1*0407-DQB1*0302 haplotype (OR 2.19, $p=0.030$). The highest risk in the study was with the HLA DRB1*1501-DQB1*0602 haplotype (OR 2.87, $p=0.005$) and this increase was greater for HPV 16 positive cases relative to controls (OR 4.78, 95%CI 1.90-11.83; $p=0.00007$). The authors suggested that based on the co-occurrence of HPV16 and DRB1*1501-DQB1*0602, the combined relative risk was 75. In addition, protective haplotypes were identified, all in the DR 13 group. These were DRB1*1301-DQB1*0603, DRB1*1302-DQB1*0604 and DRB1*1303-DQB1*0301. The DRB1*1302-DQB1*0604 among HPV 16 positive cases was found to be strong enough to be significant independently ($p=0.048$). Although the control population in the study had normal cervical smears, the study suffers from the drawback that the HPV status of the control group was not examined. It is well recognized that between 5 -50% of women with normal cervical cytology may have HPV infection, and of these up to 50% may harbour high risk or oncogenic HPV infection⁸⁵.

Nawa et al³²⁹ examined the HLA DQB1 frequency in 23 Japanese patients (age 23-35 years) with invasive squamous cell carcinoma of the cervix using a PCR-RFLP technique. Twenty patients (87%) carried a DQB1 gene encoding the HLA-DQB1*03 alleles, compared with 49.4% Japanese control subjects in the International Histocompatibility Workshop panel ($p=0.0003$). However, the correlation between DQB1*03 alleles and HPV infection was not statistically significant. The limitations of the study include the

small sample size, typing only for HPV 16 and 18, and the use of a control group that may not necessarily be comparable. By contrast, Amar et al³³⁰ investigated HLA class II in a population of 30 Jewish patients with invasive squamous cell carcinoma of the cervix and compared with 400 local healthy controls. The results showed no significant association of any of the HLA DQ alleles with cancer.

A total of three other reports were published in 1996. Sastre-Garau et al³³¹ performed PCR-SSO reverse dot blot for HLA DRB1 typing and PCR-SSO for HLA DQ typing in a population of 126 French women with invasive squamous carcinoma of the cervix. Controls were 165 randomly selected individuals previously typed for HLA DR and DQ. The results showed a decreased frequency of the DRB1*1301/02 alleles in patients (11%) compared with controls (29%) ($p=0.0004$, $OR=0.33$) and the decrease was limited to HPV positive tumors. The haplotype DRB1*1301/02-DQA1*0103-DQB1*0601 was also lower in patients (2%) than in controls (9%) ($p=0.001$, $OR=0.25$) and the decrease was again limited to the HPV positive tumours. Although the study used PCR-based strategies for HLA and HPV typing, the major drawback is the limited information on the control group, as they may not necessarily be comparable.

In a Swedish study by Allen et al³³², 150 patients with invasive squamous cell carcinoma of the cervix were examined using PCR-based HLA and HPV typing. The results were compared with data from a general Swedish population and showed the DRB1*0401-DQB1*0302 haplotype to be positively associated with disease ($p=0.05$).

In contrast to the results of the above studies, Glew et al³³³ reported no significant differences in HLA class II antigen frequencies in a group of 58 patients with squamous cell carcinoma of the cervix from Northwestern England. Further, the study showed no significant differences in the HLA antigen frequencies of patients with HPV 16 positive or

negative tumours. There were also no differences in antigen frequencies in relation to stage of disease. The control population in the study were 857 organ donors (347 males and 510 females) from the same geographical area. The relatively small patient group in the study may reflect a type -1 statistical error rather than the true biologic pattern of disease. Furthermore, the patient and control population are not necessarily comparable as the HPV status of the control group was not known. In a more recent report from Northwestern England³³⁴ an HPV 16 oncogene variant leading to an amino acid change from arginine to glycine at position 10 from the E6 consensus start codon was identified in 32% (7 out of 22) of HLA B7 - positive patients. The altered sequence was not found in HLA B7 - negative individuals. Although the substitution could have a profound effect on the interaction of the epitope to HLA B7, binding studies showed that the variant peptide binds to HLA B7 in a similar manner to its wild type equivalent. However, computer modeling suggests that the alteration may affect the amino acid residues which are exposed for interaction with the T cell receptor. This raises the possibility that both the HLA type and/or presence of mutations in specific T cell epitopes of HPV oncoproteins act in concert to determine the risk of developing cervical carcinoma or progressing from low grade SIL to cancer.

As cervical intraepithelial neoplasia occurs at a stage prior to the development of cancer and is about 50 times as common, any HLA association identified will be important in establishing the role of immunological factors in the progression to invasive cervical cancer. Indeed it has been estimated that 20 - 30 % of CIN III progress to SCC in 5 - 10 years¹¹. To date, five studies have specifically addressed this question. In a Belgian population, Vandenvelde et al³⁰³ using an HLA DQB1*03 allele-specific oligonucleotide (ASO) primed fast PCR technique, found a significant difference between normal women (174/323=0.539) and CIN I (18/24=0.750, $p=0.045$) and CIN II patients (16/21=0.762, $p=0.046$) but not CIN III (15/26=0.577, $p=0.707$). The data from the study suggest a

greater risk of high risk HPV associated dysplastic transformation of the normal cervix in DQB1*03 positive women (RR=2.647, $p=0.022$), but not a higher risk of malignant transformation (RR=1.168, $p=0.707$). This conclusion is slightly different from the results of another study by David et al³³⁵ from northwestern England which showed a higher risk of both dysplastic change as well as malignant transformation in HLA DQB1*03 positive women. Using the PCR-SSO technique, the DQB1*03 frequency in 50 patients with CIN compared with 49 age-matched controls without abnormal cervical cytology were 40% in CIN III, 22% in CIN I/II, 26% in cytology negative controls, and 21% in a local panel of blood donors (RR=2.5 for CIN III, $p=0.017$). In another report by Apple et al³⁰⁹, the frequency of distribution of HLA DR-DQ haplotypes among 128 Hispanic women with HPV 16 positive severe dysplasia was significantly different from a control population of 220 women, whereas severe dysplasia containing HPV types other than HPV 16 did not reveal any significant differences. The study also showed that the DR-DQ haplotypes previously found³⁰⁶ to be associated with HPV 16 positive cervical carcinomas were also associated with HPV 16 positive severe dysplasia/CIS.

In a Swedish study, Sanjeevi et al³³⁶ examined a population of 74 women with CIN and 164 controls using PCR-SSO for HLA DQ typing, low resolution PCR-SSP for HLA DR typing, and serological typing for HPV 16 and 6. The results showed increased risk of CIN in patients with DQB1*0602 compared with controls (OR 2.23, $p<0.01$), and the association was stronger for HPV 16 seropositive patients (OR 3.37, $P<0.05$). In addition, DRB1*15 was associated with disease (OR 2.20; $p<0.01$), stronger for HPV 16 seropositive patients (OR 5.82, $p<0.05$). The DQA1*0501-DQB1*0301 was also found to be increased among HPV seronegative patients. The study suffers significant drawbacks because cervical cytology (not biopsy) was used to diagnose CIN in the majority of cases and only 2 HPV types were tested serologically. Furthermore, serum antibodies against HPV 16 are absent in a significant proportion of patients with CIN and cancer²³².

Finally, Helland et al³³⁷, in a Norwegian population-based case-control study examined 91 patients with histologically verified CIN grade II-III and 213 control subjects. The control population were randomly selected through the Central Population Register, and were without CIN at study entry. HPV typing was performed using PCR with general nested primers, followed by type specific primers for HPV 6, 11, 16, 18, 31, 33 and X. HLA DQA1 and DQB1 typing was performed using PCR-SSO. There were no differences in frequencies of the individual alleles when the cases were compared with controls. However, the haplotype DQA1*0102-DQB1*0602 was increased in HPV positive cases (OR 3.2; $p = 0.02$) and this association was stronger for HPV 16 positive cases (OR 10.1, $p = 0.01$). It is unlikely that the discordance between the Norwegian and Swedish studies is due to genetic heterogeneity in HLA frequencies, and may reflect differences in HPV detection methodologies.

In the present study, the patients and control groups have been well characterized. The diagnosis of CIN was made by histological examination of material from women with abnormal pap smears. The control population were drawn from women attending the same clinics and had negative cytology. Furthermore, the latter population were further characterized to be negative for HPV 16, 18, 31 and 33. Thus the study comprises important subsets for analysis namely:

- (i) HPV negative and CIN negative (controls).
- (ii) HPV negative, CIN positive
- (iii) HPV positive, CIN positive.

Analysis was performed for trend as well as for direct correlation. HLA DQB1*0301 and 0302 correlated significantly with increased risk for both CIN and HPV positive CIN. DQB1*0301 had the most significant association (O.R. 2.49; $p < 0.00001$). The DR alleles that correlated significantly with increased risk of CIN were the DR4 group (O.R. 1.76,

$p < 0.001$) principally DRB1*0401 (O.R. 1.99, $p = 0.002$); DRB1*0403 (O.R. 3.61; $P = 0.02$) and DRB1*0406 (O.R. 3.74-@; $p = 0.0007$). In addition, DRB1*1101 also correlated with increased susceptibility to CIN (O.R. 2.31, $p = 0.004$). These alleles were also found to correlate significantly with CIN3 and HPV positive CIN.

DRB1*0101 and DRB1*1301 were significantly associated with protection (O.R. 0.48 and 0.67 respectively) for both HPV infection and CIN. The most significant DQB1 allele associated with protection from HPV and CIN was DQB1*0501 (O.R. 0.48, $P < 0.005$). Homozygosity at only the DQB1*0301 locus conferred an increased risk (O.R. 4.39, $p < 0.002$).

In addition, the two locus haplotypes DRB1*0401-DQB1*0301 (O.R. 2.22, $p < 0.01$), DRB1*0401-DQB1*0302 (O.R. 1.90, $p < 0.05$), DRB1*0403-DQB1*0302 (O.R. 4.34, $p < 0.01$) and DRB1*1101-DQB1*0301 (O.R. 3.95, $p < 0.003$) were significantly associated with HPV and CIN and indicated susceptibility. The haplotype which significantly correlated with protection from HPV positive CIN was DRB1*0101-DQB1*0501 (O.R. 0.48, $p < 0.01$). The significant protective and susceptibility alleles were analysed to examine for associations with individual HPV types. HPV 16 was present in 75 (57%) of cases, HPV 18 in 9 (7%), HPV31 in 17 (13%), HPV 33 in 9 (7%), and multiple HPV types were detected in 21 (16%). The best correlation was with HPV 16 and the susceptible haplotypes were DRB1*0401-DQB1*0301 (O.R. 2.63, $p < 0.02$) and DRB1*1101-DQB1*0301 (O.R. 5.80, $p < 0.001$). There was a weak positive correlation with DRB1*1101-DQB1*0301 and HPV 31 (O.R. 6.44, $p < 0.05$). The haplotype which conferred a protective effect did not show any significant correlation with any of the HPV types.

Of interest, the studies in which HLA class I frequencies were determined by serological typing in cervical cancer and controls showed an increase in HLA B12 and HLA B7^{333,255} while there was a negative association with HLA B35. HLA B12 is known to be in linkage disequilibrium with DRB1*0401-DQB1*0301²⁸⁶ suggesting that the observed increase in HLA B12 may be due to linkage disequilibrium rather than an independent effect. The negative association with HLA B35 may likewise be due linkage disequilibrium with DRB1*0101-DQB1*0501²⁸⁶, which was found to be important in the present study.

The natural history of CIN has shown that the majority of low grade lesions regress spontaneously. The plurality of HPV types associated with low grade lesions is greater than that observed in high grade lesions or in invasive cancer, suggesting the existence of HPV-type specific regression mechanisms. Taken together, the evidence from this and other studies indicate that genetic factors are involved in the control of HPV-induced tumors. Although a familial trend has not been reported in cervical cancer, a familial aggregation has been reported in about 10% of cases of epidermodysplasia verruciformis, a disease characterized by a high susceptibility to cutaneous HPV³³⁸. The study of immunogenetic mechanisms controlling the regression or the development of genital neoplasia should shed light on mechanisms involved in the progression or regression of HPV-associated tumors.

6.3 MECHANISMS OF HLA CLASS II ASSOCIATION WITH HPV AND CIN

The significance of HLA association with cervical cancer is supported by data on the analysis of HLA class I and II using restriction fragment length polymorphisms in New Zealand rabbits infected with Shope cotton-tail rabbit papillomavirus, which showed a strong linkage between wart regressions and DR locus, and an increased risk of malignant transformation with the DQ locus³⁰⁵. A number of diseases have been associated with the

DQB1*03 group of alleles. These include autoimmune disorders as well as malignancies. The former group include the lupus-anticoagulant response³³⁹, vitiligo³⁴⁰, ocular cicatricial pemphigoid³⁴¹, and the herpes-associated form of erythema multiforme³⁴². Malignant diseases associated with HLA DQB1*0301 include malignant melanoma²⁶¹, adult T-cell leukemia, T-cell lymphoma, human T-cell leukemia virus type 1 carrier state³⁴³ and gastric adenocarcinoma³⁴⁴.

There are at least three possible ways by which the association between DQB1*03 and HPV positive disease may be explained. Firstly, these women may present peptide antigen to CD4+ T cells ineffectively; secondly there may be clonal deletion of antigen specific T cell during thymic maturation may occur; or thirdly there may be active suppression of immune response to HPV in DQB1*03 positive women. Indeed, there is a high level of expression of HLA DQ in the thymic cortex³⁴⁵ and a role for negative selection for HPV specific T cell clones would fit predisposition to HPV positive CIN by the DQB1*03 alleles.

The other possible mechanism is based on observations of HLA associated immunological low responsiveness to antigens such as streptococcal cell wall³⁴⁶, schistosoma³⁴⁷, mycobacterium leprae³⁴⁸, tetanus toxoid³⁴⁹ and hepatitis surface antigen³⁵⁰ either after natural exposure or after vaccination. Despite the controversy regarding the function of suppressor T cells, there is evidence to suggest that HLA DQ maybe the preferred restriction element for immunological suppression mediated by CD8+ T suppressor cells³⁵¹⁻³⁵². It is possible that women who are positive for the DQB1*03 phenotype maybe unable to mount an effective cytotoxic T cell response against HPV infection. This is particularly important as it has been shown that HPV16 E7 is a target for cytotoxic T cells and to mediate tumour rejection³⁰⁷.

There are several lines of evidence that cloned T suppressor (Ts) cells express conventional α and β genes³⁵³⁻³⁵⁴. These clones respond to peptides presented in the context of MHC class I or II molecules. There are a number of ways by which this class of T cells can suppress immune responses in an antigen-specific manner. The first mechanism involves soluble antigen specific factors. These factors are comprised, at least in part, of some form of TCR α - and/or β chains³⁵⁵. A second mechanism of suppression is cytotoxicity, which requires specific recognition of antigen-MHC complexes and is achieved by specific (cell mediated) or non-specific (cytokine-mediated) means. For example, it has been shown that CD8+ T cells can kill a CD4+ T cell line that mediates experimental autoimmune encephalomyelitis and neutralize their ability to mediate disease in vivo³⁵⁶, and a Ts clone that kills T helper (Th) cells has been described³⁵⁷. Specific suppression could be achieved by killing T cells bearing clonally distributed TCRs (via recognition of TCR-peptide-MHC complexes).

In the case of HLA DR associations with HPV infection this may be due to polymorphisms in the second exon of the molecule. For example, position 86 of the β chain is dimorphic and the amino acids glycine (Gly) and valine (Val) are found at this position. The functional significance of the Gly/Val dimorphism at this position has been explained by the resolution of the tertiary structure of HLA DRB1*0101. β 86 contributes to the formation of the 'hydrophobic' pocket and its substitution by valine restricts the size of the peptide side chain which can bind to HLA DRB1*0101 and therefore the peptide which can bind to the T cell receptor. A possible explanation for the protective effect of HLA DRB1*0101 in this study is that an immunodominant HPV epitope might contain a large hydrophobic side chain (Trp, Tyr, Phe, Leu, Ileu) as a major anchor in the β 86 Gly pocket and this epitope binds with high affinity to DRB1*0101.

6.4 POLYMORPHIC STRUCTURAL FEATURES OF HLA-DQ MOLECULES ASSOCIATED WITH SUSCEPTIBILITY OR RESISTANCE TO HPV ASSOCIATED CIN

An attractive hypothesis for the molecular basis of the association between HLA DQ and HPV associated CIN may be based on the results of motif analysis in chapter 5: HPV epitopes bind with higher affinity to the resistant than to susceptible DQ molecules, leading to a more effective elimination of the virus. Although this hypothesis is not contrary to current immunological paradigms, very little is known about the function of DQ molecules. They are only constitutively present on a subfraction of antigen-presenting cells, and at much lower density than DR molecules³⁵⁸. However, their expression on APCs can be induced by gamma interferon³⁵⁹, and infectious agents such as Epstein Barr Virus³⁶⁰ and Human Papillomavirus³⁶¹. It has been postulated that DQ molecules exercise epistatic action over DR molecules³⁶² and that they are the mediators of immunosuppression³⁵¹.

In order to examine this hypothesis of differential binding to susceptibility and protective alleles, a model of the structure of HLA DQ molecules based on the HLA DR1 structure can be inferred³⁶³. This model suggests that they possess the mould of a class II histocompatibility molecule with an antigen-binding groove in the $\alpha 1\beta 1$ domain that is bounded by a "floor" of eight β -sheets and two "walls" of antiparallel α -helices. There is an $\alpha 2\beta 2$ domain that contains the homodimerisation region, the CD4-binding area and the Arg-Gly-Asp loop. This domain and the $\alpha 1\beta 1$ domain dimerise in some alleles with their counterparts in an identical DQ molecule forming a homodimer of $\alpha\beta$ heterodimers.

The polar residues lining the antigen binding groove of DR1 and participating in hydrogen bonding with antigenic peptide are located in exactly the same positions in modeled molecules¹⁶⁴⁻¹⁶⁵, with nearly identical orientations³⁴⁸. Within the binding groove are five pockets which can trap specific residues of antigenic peptides. The first pocket of DQ

molecules (formed by $\alpha 10$, 27, 34, 35, 46 and $\beta 85$, 86, 89 and 90) is either amphiphilic or hydrophilic, as judged from the amino acids that line-up this formation. The hydrophilic variant of this pocket appears shallower, because of the presence of bulky residues in $\beta 85$, 86 and 89 (Leu, Glu and Thr instead of Val, Ala, and Gly respectively for the amphiphilic variant). The character of the first pocket is also modified by residue $\alpha 34$ (Glu or Gln). Thus, the HLA DQB1*0501, which is protective for HPV associated CIN in this study has valine, alanine and glycine in the $\beta 85$, 86 and 89 positions respectively and is therefore amphiphilic. By contrast, the susceptibility alleles HLA DQB1*0301, 0302 and 0303 have leucine, glutamate and threonine in the same positions and are therefore hydrophilic.

The second HLA DQ pocket appears to be the most prominent or anchoring pocket. This is probably due to the presence of small residues in position $\alpha 9$ and $\beta 13$ of DQ molecules in contrast to the bulky glutamine or phenylalanine respectively in DR1. There is extensive polymorphism in the four residues from the $\beta 1$ helix of this pocket (70, 71, 74 and 78). In the case of HLA DQB1*0501, which correlates with resistance to HPV associated CIN, these residues consist of glycine, alanine, serine and valine respectively. All the DQB1*03 susceptibility alleles have arginine, threonine, glutamate and valine in these positions and the size of these residues may restrict peptide binding to an immunodominant HPV epitope.

The presence of aspartate in $\beta 57$ which is part of the fifth pocket is a major determinant of peptide affinity³¹⁸. This residue consists of valine in HLA DQB1*0501. HLA-DQB1*0302 and DQB1*0303 differ only in Ala to Asp polymorphism at codon 57, whereas DQB1*0301 encodes Asp 57 and three additional polymorphisms at positions 13, 26, and 45. The aspartic acid residue on DQB1*0301 and 0303 likely interacts with an arginine at residue 79 of the DQA1 chain to form a salt bridge by analogy with a similar structure of HLA-DR1¹⁶⁵. Although an influence of this potential salt bridge has been

suggested^{322,364}, it does not appear to significantly influence the HLA-DQ3 association with HPV infection and CIN since all three alleles result in increased risk of disease.

There are important differences in the β 49-56 dimerisation patch¹⁶⁴ of all the HPV associated susceptible DQ molecules when compared with the patch of the DQB1*0501 resistant molecule. In the DR molecule, there is a monomorphic dimerisation patch, with dimerisation probably promoted after T cell receptor binding¹⁶⁴, by symmetrical salt bridges between β 52Glu of one heterodimer and β 55Arg of the opposite heterodimer. By contrast DQ alleles are polymorphic in this region leading to β 49-56 sequences that may be very hydrophobic, amphiphilic or hydrophilic. The alleles DQB1*0301, 0302 and 0303 have a hydrophilic patch. On the other hand, the protective DQB1*0501 allele is amphiphilic. It contains an arginine at position 55, and glutamine in position 53 and proline at position 56, right opposite each other at the first turn of the β 1 helix. The relative ease of homodimerisation by the protective DQB1*0501 molecule means that in case any cognate T-cell clones exist in the periphery, their activation upon recognition of this protective DQ molecule complexed with an HPV peptide would be easy. By contrast, susceptible DQ molecules will form homodimers with more difficulty leading to less effective activation of cognate T-cell clones. In the case of the α 2 β 2 homodimerisation domain, it involves a large surface area of the DQ molecule and the dimerisation is stabilized by multiple interactions involving charged and hydrophobic residues^{164,363}. There appears to be no difference between susceptible and protective DQ molecules in this domain.

The CD4 binding area is formed by the homodimerisation of DQ molecules (β 2 of one DQ heterodimer to α 2 of another DQ heterodimer), and has been shown in DR to be composed of the sequence β 134-148 and several residues on the alpha chain apposed to this sequence. Of the residues shown to be critical for CD4 binding to HLA DR β , by site directed mutagenesis (β 137Glu, 142Val, 143Val)³⁶⁵, all remain invariant in the DQB

alleles suggesting that this region of the DQ molecule is unlikely to be important in determining susceptibility or resistance to HPV.

The Arg-Gly-Asp loop on β 167-169, present in the protective allele, HLA DQB1*0501, is absent in HLA DQB1*0301 where β 167 is histidine. The exact function of this RGD loop in DQ molecules is unknown but probably functions in cell adhesion as in other integral membrane proteins and proteins of the extracellular matrix involved in such function³⁶⁶⁻³⁶⁷, and may be important in the DQ restricted T-cell clone activation.

A scheme where the three structural features of the DQ molecules segregate in the two phenotypes of susceptible and protective HLA-DQB alleles that confer susceptibility or protection to HPV-associated CIN is shown in table 6.1. The difference in the physicochemical properties of the antigen-binding groove of susceptibility and protective DQ alleles would translate into different affinities for an "immunodominant" epitope in HPV. Such differences would certainly play a role both in the ontogenesis of the immune system and in the mounting of a specific DQ restricted immune response in the mature organism. Indeed, the human embryonic thymus is very rich in DQ molecules that probably function as restriction elements³⁶⁸. Therefore, the CD4+ T-cell clones recognizing the combination of susceptible DQ molecules with its bound peptide epitope would be eliminated. In the periphery, the susceptible DQ molecules expressed under proper stimulation on antigen presenting cells could bind to HPV derived peptide(s) and present such complexes to cognate CD4+ T cells. The ensuing immune reaction may be insufficient for viral clearance.

The dominant effect of the protective DQ molecule in the periphery could be exercised in the same manner. The differences in the physicochemical character of the antigen-binding groove assures preferential peptide binding. The difficulty of dimerization by the

susceptibility molecules ensures that even though the peptide has been trapped, the activation of cognate T-cell clones that might have escaped elimination in the thymus would be very difficult.

It is to be noted that in offering an explanation for the involvement of HLA DQ molecules in the susceptibility to HPV-associated CIN based on HLA structural features, account is not taken of the polymorphisms of DQ molecules in the intracellular amino acid sequences that participate in signal transduction³⁶⁹, or the possible differences in the level of expression of various DQ alleles. Also, a number of regulatory sequences have significant effects on DQB genes³⁷⁰ and their possible role in the HPV induced cervical carcinogenesis is unknown.

| Domain | Features | Character | DQB1*03 (Susceptible) | DQB1*0501 (Protective) |
|-------------------|-----------------------------------|---|------------------------------|---------------------------|
| $\alpha 1\beta 1$ | Antigen binding groove | Polymorphic | | |
| | First residue binding pocket | Dimorphic, hydrophilic, amphiphilic | Hydrophilic | Amphiphilic |
| | Residue at $\beta 57$ | Polymorphic | 0301 Asp, 0302 Ala, 0303 Asp | Valine |
| | $\beta 49$ -55 dimerisation patch | Polymorphic, very hydrophilic, amphiphilic, hydrophilic | | Amphiphilic |
| $\alpha 2\beta 2$ | CD4 binding region | | No discernible differences | |
| | $\beta 167$ -169 RGD loop | Probably involved in cell adhesion | Absent in 0301 | Present |

Table 6.1: Summary of the structural features of HLA DQB1*03 and HLA DQB1*0501.

6.5 CLINICAL IMPLICATIONS : HLA TYPING AND VACCINE DEVELOPMENT

This study raises the question as to whether women infected with HPV will benefit from HLA typing to predict disease susceptibility and/or severity. At the present, there is no data to suggest that information on HLA type will alter current clinical practice. However, the information would be useful in a research setting in screening programmes, evaluation of treatment outcome (surgery, radiation therapy, and chemotherapy), on-going vaccination trials as well in the design of novel immunomodulatory strategies for the prevention and treatment of HPV associated cervical cancer.

Screening with the Papanicolaou smear remains the best available method of reducing the incidence and mortality of invasive cervical cancer. There are large numbers of women with Papanicolaou smears showing squamous intraepithelial lesions (SIL) each year. Only a minority of these women will progress to invasive cancer, and it would be advantageous to develop predictive markers to identify those women. The need for predictive markers is even more important in the category of patients with atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS) and low-grade SIL (LGSIL), lesions that are usually managed expectantly. However, Cox et al³⁷¹, Wright et al³⁷² and Kinney et al³⁷³ reported that 6.9%, 6.1% and 7.3% of women with ASCUS cytology, respectively harboured histologic high-grade SIL (HGSIL). Attempts to improve the triage of these women with HPV typing assays have yielded conflicting results. The addition of HLA class II typing may allow "low-risk" women (with ASCUS, AGUS, LGSIL or HGSIL) to avoid costly and potentially morbid diagnostic and therapeutic procedures. Further, women in the different categories could be followed longitudinally over several years to determine the effects of HLA type on the natural history of disease.

Another major clinical impact of this study is likely to be in the area of vaccine design for HPV associated CIN and cervical cancer. The rationale for the use of HPV epitopic determinants as prophylactic and therapeutic cancer vaccines is supported by the following:

- i. In a recent study utilizing the sorting signal of the lysosomal-associated membrane protein-1 (LAMP-1) to reroute HPV 16-E7 into the MHC class II processing pathway, there was enhanced presentation to CD4+ cells, greater E7 specific lymphoproliferative activity, antibody titres, and CTL activity³⁷⁴.
- ii. Using the LAMP-1 / HPV E-7 chimera expressed in a recombinant vaccinia virus, Lin et al³⁷⁵ showed that 80% of vaccinated mice remained tumour free 3 months after injection compared to progressive tumour growth in all wild type E7 injected mice. Further, vaccination cured mice with small established tumours, whereas the wild type E7 vaccinia showed no effect on established tumour.
- iii. It has been recognized that in the case of other tumours, especially human melanoma, systemic administration of melanoma-associated antigens (MAA) derived peptides can elicit anti-tumor CTL activity in-vivo³⁷⁶⁻³⁷⁷.

As an increasing number of HPV 16 and 18 epitopes are reported, the practical question to be raised is which, given limited resources, should be given priority for clinical trials. The identification within the context of a specific HLA restriction element of the *immunorelevant* antigen among the repertoire of several possible HPV peptide molecules may allow a more focused selection of the most appropriate target antigen for vaccination. The HPV peptide epitopes identified in this study (chapter 5) as probably being capable of binding with high affinity to both susceptibility and protective alleles may be utilized in in-vitro and in-vivo vaccine design experiments using the LAMP-1 sorting signal to route the peptides into the HLA class II pathway.

The processing of endogenous HPV proteins for class II restricted presentation is of considerable practical interest because it allows direct recognition of HPV infected cells by CD4+ cells. Although CD4+CD8- T cells are often referred to as belonging to the helper/inducer subset, this population is heterogeneous in terms of their effector functions such as lymphokine production and secretion or cytotoxicity. In humans, CD4+ cytotoxic T cells have been described as an effector population in a variety of viral infections including EBV³⁷⁸, hepatitis B³⁷⁹ and herpes simplex³⁸⁰. Furthermore, CD4+ T cells have been shown to be critical in generating immune responses against several solid malignancies in murine³⁸¹⁻³⁸² and human systems³⁸³⁻³⁸⁴. Given that an appropriate peptide epitope in association with HLA molecules might be expressed on the surface of infected cells, lysis of these cells by CD4+ cytotoxic T cells is likely to be an important mechanism for the protection against persistent HPV infection.

The advantages of peptide vaccines include stability, ease of preparation, transportation, and injection; and they do not pose the biological risks that may occur with the use of intact proteins. The potential disadvantages include the requirement for knowledge of the epitopic determinant for each HLA allele, the potential limitation of the targeting of only one restriction element among several expressed by a given tumour, and dependence for immunogenicity on the stability of the peptide/HLA complex³⁸⁵. Although the use of peptide mixtures that will bind to several class I alleles may overcome some of these problems, the immunogenicity of these mixtures will need to be determined. Since MHC class II peptide binding exhibit allele specificity as well as promiscuity, knowledge from studies of class II association with HPV infection should lay the framework for the development of "promiscuous" immunogenic peptides that would be presented via the class II pathway.

Vaccination trials in mice and rats have clearly demonstrated the feasibility of inducing immunity that can protect against the growth of HPV 16 containing tumours. A number of immunization approaches have been used with varying degrees of success. It was shown that immunization of C3H/HeN mice with syngeneic fibroblasts transfected with HPV 16 E7 gene conferred protection against E7 transfected syngeneic tumour cells³⁰⁷. Similarly, immunization of mice with HPV 16 gene transfected fibroblasts induced regression of transplanted tumours expressing E6³⁸⁶. Populations of CTL isolated from the spleens of mice which rejected the tumour challenge were shown to specifically lyse E6 expressing target cells *in vitro*. Meneguzzi et al³⁸⁷ used recombinant vaccinia virus expressing E6 or E7 to immunize rats which were then challenged with cells co-transformed with HPV 16 and Ras. The study showed tumour development to be delayed or prevented in immunized rats. Vaccines based on recombinant live virus have the advantage of physiologic antigen delivery and is not HLA haplotype dependent since different HLA alleles will select different peptides from the naturally processed peptide pool. The major disadvantage is safety concern but this may be obviated by designing vaccines with non transforming mutant variants without compromising the immunogenicity of the parent protein.

In another approach, synthetic peptides corresponding to residues 49-57 of the E7 protein were used to immunize C57BL/6 mice and they showed complete or partial protection against tumour formation by transformed cells containing HPV 16 and Ras³⁸⁸. The immunogenicity of HPV 16 E6 and E7 proteins were analyzed extensively by Kast et al³⁸⁹. A set of 240 nonamer peptides derived from E6 and E7 were synthesized and tested for binding to several of the most common HLA-A alleles. From these studies, a number of high affinity binding peptides were determined and the immunogenicity of these peptides was tested *in vivo* by immunization of HLA-A2.1⁺ transgenic mice and *in vitro* by stimulation of CTLs from normal HLA-A2.1⁺ human peripheral blood lymphocytes³⁹⁰. Four high-affinity binding peptides were immunogenic in the transgenic mice and three of

these peptides were also immunogenic to CTLs from normal donors. Human HLA-2.1-restricted CTL clones specific for these peptides were able to recognize and lyse peptide-pulsed targets as well as HLA-A2.1+ cervical carcinoma cell line CaSki that expresses the HPV-16 E6 and E7 genes. These results suggest that these peptides are naturally processed T cell epitopes of HPV-16 and may act as cervical carcinoma tumour antigens.

There are at least six human papillomavirus vaccine trials that have been initiated worldwide in the past 12 months. Although the growing number of HPV vaccine trials has raised hopes for the future of vaccine therapy in cervical cancer, differences among the trials make the details of that future still far from clear. All of the trials are small phase I or phase I/II studies, and are all testing vaccines against HPV 16 and 18. There is however an array of different vaccine formulations and a variety of patients, as seen in the following summary of the studies:

1. National Cancer Institute (NCI) phase I trial of HPV 16 E7 lipopeptide vaccine for recurrent or refractory cervical cancer. In this study, a vaccine consisting of a lipidated HPV E7 peptide epitope (Cytel Corporation, San Diego) linked to a nonspecific helper peptide (PADRE) is used in HLA-2 and HPV 16-positive patients with recurrent or refractory cancer.
2. NCI phase II pilot study of HPV 16 E6 and E7 peptide vaccines for advanced or recurrent cervical cancer. The trial involves the use of antigen-presenting cells pulsed with synthetic peptide corresponding to the tumour's HPV 16 E6 or E7.
3. Multicenter European trial using a vaccine as an adjunct to surgery and radiation therapy in women with early stage invasive disease.
4. Two HPV vaccine trials, one at the University of Wales in Cardiff and the other at the Norris Cancer Center of the University of Southern California, Los Angeles - will give HPV vaccines to women with high grade preinvasive lesions (CIN2/3).

In the future, the development of a prophylactic or therapeutic vaccine for cervical cancer may offer an attractive and cost-effective immunologic approach to reduce the need for expensive screening and surveillance prevention programs and substantially decrease the worldwide morbidity from this disease.

6.6 CONCLUSIONS

Genetic variation at the HLA loci accounts for differences in immune recognition between individuals and similarly underlies differences in disease susceptibility to HPV associated CIN. One of the important functional consequences of this genetic variation is the generation of distinct patterns of peptide recognition and antigen presentation. Understanding the structural basis for these functional properties of specific HLA molecules is helping to unravel the peptide-binding properties that are inherent to each distinct allele. In studying HLA class II genes with HPV associated CIN, these peptide-specific interactions presumably form the basis for genetically regulated events in immune activation and disease.

There is compelling evidence to suggest that the HLA class II type is important in determining the risk of HPV infection and progression to CIN and cancer. Taken together, the most consistent finding in several studies is the increased risk of HPV infection, CIN and cancer in individuals with HLA DQB1*03. A number of other HLA class II alleles have been shown to correlate with susceptibility or protection in different populations. It is possible that differences in results are either due to variations in methodologies employed in the different studies or different patterns of linkage disequilibria with the disease susceptibility gene in different populations. Difficulties of single mechanisms to explain HLA association with HPV and cervical cancer is to be expected, since the development of cancer is a complex process influenced by many factors, environmental and genetic. Nevertheless, all these studies should add a new insight into the development of

immunomodulatory strategies for the prevention and treatment of cervical cancer. Several vaccination approaches against HPV infection are currently being evaluated and it is expected that further refinements in vaccine design and delivery will be made based on rapidly emerging information on the role of HLA class II in HPV infection.

6.7 FUTURE STUDIES

6.7.1 HLA ASSOCIATION STUDIES

While ethnic variations in HLA haplotype frequencies may explain the differences between the Mexican-American and African-American patient cohorts on the one hand, and the European patient cohorts on the other, the heterogeneity on HLA frequencies among the North European populations is hardly sufficient to explain the observed differences in association reported for English, German, Norwegian and Swedish patients. The differences may really be due to statistical error or heterogeneity in an as yet undetermined genetic or environmental fashion, including the HPV genome. The former possibility can be addressed by replicating the analysis in an unrelated set of patients.

The possible susceptibility and protective haplotypes identified in this study need to be confirmed in a larger sample size especially if relations to specific HPV types are to be determined. Additional studies on patients with invasive cervical cancer are also needed to determine the contribution of HLA class II alleles in progression to invasive cancer. A complete HLA class I typing of cases with CIN will allow the identification of the complete haplotype and also determine the contribution of individual alleles towards susceptibility and protection.

6.7.2 ASSOCIATION WITH HLA RELATED GENES AND P53

Differences in the distribution of HLA class II genes observed after a comparison of patients and controls may suggest that the immune response to HPV may be determined, at

least in part, by specific class II alleles. However, these differences could be related to a linkage disequilibrium with other MHC-related genes such as TAP-1, the TNF α gene promoter, or antigen processing regulator genes. Furthermore, recently, it was shown that patients with HPV associated tumors have an overrepresentation of homozygous arginine-72p53 compared with the normal population³⁹¹. This finding will need to be confirmed in larger populations and in different geographic regions to determine the combined roles of HLA and p53 polymorphisms on HPV associated cervical carcinogenesis.

6.7.3 IMMUNODOMINANCE AND ANTIGEN PRESENTATION

The ability of the immune system to direct T-cell responses against a select number of peptides is termed immunodominance³⁹². Epitopes that trigger potent T-cell activation and proliferation are classified as immunodominant. By contrast, epitopes that are poor activators of cellular immune response are termed subdominant, whereas those peptides that fail to elicit any response are cryptic. These terms indicate that there may be a discrepancy between the number of peptides within an antigenic protein that could be predicted to potentially bind to a particular HLA molecule and the number of epitopes actually recognized in a CTL response to that protein. The molecular events that control immunodominance appear to be complex with both APC and T cells regulating the process. Since the binding affinity of naturally processed peptides for class II proteins plays a significant role in influencing the hierarchy of epitopes displayed to T cells³⁹², in-vitro studies of the binding affinity of predicted motifs of L1, L2, E6 and E7 may be used to select a library of peptides for evaluation of CTLs from normal human peripheral blood lymphocytes. In addition, the peptides could be tested *in vivo* by immunization of HLA DQ and DR transgenic mice.

6.7.4 VACCINATION TRIALS

The expression of HPV E6 and E7 genes is constitutive in cervical tumors and required for the maintenance of the transformed state. Because of their continued expression in tumor cells, the E6 and E7 proteins are promising targets for immune intervention. Immunodominant epitopes from E6 and E7 identified as above (6.7.3) could be used in clinical trials for the treatment of HPV associated cervical cancer. On the other hand, immunodominant epitopes from L1 and L2 could be used in the clinical trials for prevention and treatment of HPV associated CIN. In this way, HPV vaccine design will be based on a firm knowledge of the HPV epitopes involved in antigen processing and presentation to T lymphocytes.

REFERENCES

1. Parkin DM, Laara E, Muir CS. Estimates of the worldwide frequency of sixteen major cancers in 1980. *Int. J Cancer* 1988;41:184.
2. Muir C, Waterhouse J, Mack T, et al. *Cancer Incidence in five continents*. IARC Scientific Publications 1987;V.
3. Wingo PA, Tong T, Bolden S. *Cancer Statistics, 1995*. *Ca Cancer J Clin* 1995;45:8-30.
4. Coppleson M, Reid BL. Interpretation of changes in the uterine cervix. *Lancet* 1967;2:216-217.
5. Richart RM. Natural history of cervical intra-epithelial neoplasia. *Clin. Obstet. Gynaecol.* 1968;10:748.
6. Richart RM. Causes and management of cervical intraepithelial neoplasia. *Cancer* 1987;60:1951-1959.
7. Robertson AJ, Anderson JM, Swanson Beck J. Observer variability in histopathological reporting of cervical biopsy specimens. *J. Clin. Path.* 1989;42:231-238.
8. NCI. The revised Bethesda system for reporting cervical/vaginal diagnoses: Report of the 1991 Bethesda workshop. *JAMA* 1992;267:1892.
9. Campion MJ, Cuzick J, McCance DJ, Singer A. Progressive potential of mild cervical atypia: prospective, cytological, colposcopic and virologic study. *Lancet* 1986;1:237-240.
10. Nasiell K, Nasiell M, Vaclavinkova V. Behavior of moderate cervical dysplasia during lon-term follow - up. *Obstet. Gynecol.* 1983;61:609-614.
11. Chang AR. Carcinoma in situ of the cervix and its malignant potential : a lesson from Newzealand. *Cytopathology* 1990;1:321-328.
12. Boring CC, Squires TS, Tong T. *Cancer statistics*. *CA-Cancer J. Clin.* 1994;44:7.
13. Kjorstad KE. Adenocarcinoma of the uterine cervix. *Gynecol. Oncol.* 1977;5:219.
14. Berek JS, Hacker NF, Fu YS, et al. Adenocarcinoma of the uterine cervix: histologic variables associated with lymph node metastasis and survival. *Obstet. Gynecol.* 1985;65:46.
15. Hopkins MP, Morley GW. A comparison of adenocarcinoma ans squamous cell carcinoma of the cervix. *Obstet. Gynecol.* 1991;77:912.
16. Shingleton HM, Gore H, Bradley DH, Soong SJ. Adenocarcinoma of the cervix. I. Clinical evaluation and pathologic features. *Am. J. Obstet. Gynecol.* 1981;139:799.
17. Kilgore LC, Soong SJ, Gore H, et al. Analysis of prognostic features in adenocarcinoma of the cervix. *Gynecol. Oncol.* 1988;31:137.
18. Stern R. Fatti statistiche relativi alla malattie cancerose. *Giornale per servise al progesi della pathologia della terapeutica* 1842;2:507-517.
19. Fraumeni J, F. Jr., Lloyd JW, Smith EM, Wagoner JK. Cancer mortality among nuns : role of marital status in etiology of neoplastic disease in women. *Journal of the National Cancer Institute* 1969;42:455-468.
20. Boyd JT, Doll R. A study of the aetiology of carcinoma of the cervix uteri. *Br. J. Cancer* 1964;18:419-434.
21. Jones EG, MacDonald I, Breslow L. A study of epidemiologic factors in carcinoma of the uterine cervix. *Am. J. Obstet. Gynecol.* 1958;76:1-10.
22. Kessler II, Kulcar Z, Zimolo A, Grgurevic M, Strnad M, Goodwin J. Cervical cancer in Yugoslavia. II. Epidemiologic factors of possible etiologic significance. *J. Natl. Cancer Inst.* 1974;53:51-60.
23. Pridan H, Lilienfeld AM. Carcinoma of the cervix in Jewish women in Israel, 1960-67 : An epidemiologic study. *Isr. J. Med. Sci.* 1971;7:1465-1470.
24. Brinton LA, Hamman RF, Huggins GR, et al. Sexual and reproductive risk factors for invasive squamous cell cervical cancer. *J. Natl. Cancer Inst.* 1987;79:23-30.

25. Peters RK, Thomas D, Hagan DG, Mack TM, Henderson BE. Risk factors for invasive cervical cancer among latinas and non-latinas in Los Angeles County. *J. Natl. Cancer Inst.* 1986;77:1063-1077.
26. Singer A. The uterine cervix from adolescence to the menopause. *Br. J. Obstet. Gynaecol.* 1975;82:81-89.
27. Brinton LA. Epidemiology of cervical cancer - overview. In: Munoz N, Bosch FX, Shah KV, al e, eds. *The epidemiology of cervical cancer and human papillomavirus.* Oxford: Oxford University Press, 1992.
28. Franco EL. Viral etiology of cervical cancer: A critique of the evidence. *Rev. Infect. Dis.* 1991;13:1195-1206.
29. zur Hausen H. Condylomata acuminata and human genital cancer. *Cancer Res.* 1976;36:794.
30. Gissman L, de Villers EM, zur Hausen H. Analysis of human genital warts (condylomata acuminata) and other genital tumors for human papillomavirus type 6. *Int. J. Cancer* 1982;29:143-146.
31. Gissman L, zur Hausen H. Physical characterization of the deoxyribonucleic acids of different human papillomaviruses (HPV). *Med. Microbiol. Immunol.* 1978;166:3-11.
32. Gissman L, zur Hausen H. Partial characterization of viral DNA from human genital warts (condylomata acuminata). *Int. J. Cancer* 1980;25:605-609.
33. Coggin JR, Zur Hausen H. Workshop on papillomaviruses and cancer. *Cancer Res.* 1979;39:545-546.
34. Chow LT, Reilly SS, Broker TR, Taichman LB. Human papillomavirus types 6 and 11 mRNAs from genital codylomata acuminata. *J. Virol* 1987;61:2581-2588.
35. de Villers EM. Hybridization methods other than PCR: an update. In: Munoz N, Bosch FX, Shah KV, Meheus A, eds. *The epidemiology of human papillomavirus and cervical cancer.* Oxford: Oxford University Press, 1992.
36. Lorincz AT, Reid R, Jenson B, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk association of 15 common anogenital types. *Obstet. Gynecol.* 1992;79:328-337.
37. Lambert PF. Papillomavirus DNA replication. *J. Virol* 1991;65:3417-3420.
38. Schwarz E, Freese UK, Gissman L, et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cell lines. *Nature* 1985;314:111.
39. Clertant P, Seif I. A common function for the polyoma virus large T and papillomavirus E1 proteins? *Nature* 1984;311:276-279.
40. Lusky M, Botchan MR. Characterization of the bovine papillomavirus plasmid maintenance sequences. *Cell* 1984;36:391-401.
41. Romanczuck H, Howley PM. Disruption of either the E1 or the E2 regulatory gene of human papillomavirus type 16 increases viral immortalization capacity. *Proc. Natl. Acad. Sci. USA* 1992;89:3159.
42. Ustav M, Stenlaud A. Transient replication of BPV-1 requires two viral polypeptides encoded by the E1 and E2 open reading frames. *EMBO J.* 1991;10:449-457.
43. Ustav M, Ustav E, Szymanski P, et al. Identification of the origin or replication of the bovine papillomavirus and characterization of the viral origin recognition factor E1. *EMBO J.* 1991;10:4321-4329.
44. Blitz I, Laimins LA. The 68-Kd E1 protein of BPV-1 is a DNA binding phosphoprotein which associates with the E2 transactivator in vitro. *J. Virol* 199;65:649-656.
45. Wilson V, Ludes -Meyers J. A BPV E1-related protein binds specifically to BPV DNA. *J. Virol.* 1991;65:5314-5322.
46. Mohr I, Clark R, Sur Sea. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. *Science* 1990;250:1694-1699.

47. McBride AA, Schlegel R, Howley PM. The carboxy-terminal domain shared by the bovine papillomavirus E2 transactivator and repressor proteins contains a specific DNA binding activity. *EMBO J.* 1988;7:533-539.
48. Lazo PA, DiPaolo JA, Popescu NC. Amplification of the integrated viral transforming genes of human papillomavirus 18 and its 5' flanking cellular sequence located near the myc protooncogene in HeLa cells. *Cancer Res.* 1989;49:4305-4310.
49. Thierry F, Yaniv M. The BPV1-E2 transacting protein can be either an activator or repressor of the HPV-18 regulatory region. *EMBO J.* 1987;6:3391.
50. Romannczuk H, Thierry F, Howley PM. Mutational analysis of the cis-elements involved in E2 repression of the HPV-16p97 and HPV-18p105 promoters. *J. Virol.* 1990;64:2489.
51. Doorbar J, Campbell D, Grand RJA, Gallimore PH. Identification of the human papillomavirus 1a E4 gene product. *EMBO J.* 1986;5:355-362.
52. Doorbar JS, Ely S, Sterling J, McLean C, Crawford L. Specific interaction between HPV 16, E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature* 1991;352:824.
53. Leptak C, Ramon y Cajal S, Kulke R, et al. Tumorigenic transformation of murine keratinocytes by the E5 genes of bovine papillomavirus type 1 and human papillomavirus type 16. *J. Virol.* 1991;65:7078.
54. Leechenachi P, Banks L, Moreau F, Matlashewski G. The E5 gene from human papillomavirus type 16 is an oncogene which enhances growth factor-mediated signal transduction to the nucleus. *Oncogene* 1992;7:459-465.
55. Baker CC, Phelps WC, Lindgreen V, Braun MJ, Gonda MA, Howley PM. Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. *J. Virol* 1987;61:962-971.
56. Tsunokawa Y, Takebe N, Kasamatsu T, Terada M, Sugimura T. Transforming activity of human papillomavirus type 16 DNA sequence in a cervical cancer. *Proc. Natl. Acad. Sci. USA* 1986;83:2200-2203.
57. Watanabe S, Kanda T, Yoshiike K. Human papillomavirus type 16 transformation of primary human embryonic fibroblasts requires expression of open reading frames E6 and E7. *J. Virol.* 1989;63:965-969.
58. Munger K, Phelps WC, Bubb V, Howley PM, Schlegel R. The E6 and E7 genes of the human papillomavirus are necessary and sufficient for transformation of primary human keratinocytes. *J. Virol.* 1989;63:4417.
59. Sedman SA, Hubert N, Vass WC, et al. The full length E6 protein of human papillomavirus type 16 has transforming and trans-activating activities and cooperates with E7 to immortalize keratinocytes in culture. *J. Virol.* 1991;65:4860-6.
60. Bedell MA, Jones KH, Grossman SG, et al. Identification of human papillomavirus type 18 transformation genes in immortalized and primary cells. *J. Virol.* 1989;63:1247-1255.
61. Halbert C, Demers G, Galloway D. The E6 and E7 genes of HPV 6 have weak immortalization activity in human epithelial cells. *J. Virol.* 1992;66:2125-2134.
62. Hudson JB, Beddell MA, McCance DJ, Laimis LA. Immortalization and altered differentiation of human keratinocytes in vitro by the E6 and E7 open reading frames of papillomavirus type 18. *J. Virol.* 1990;64:519.
63. Hurlin PJ, Smith PP, Perez-Reyes N, Blanton RA, McDoughall JK. Progression of human papillomavirus type 18-immortalized human keratinocytes to a malignant phenotype. *Proc. Natl. Acad. Sci. USA* 1991;88:570-574.
64. Cripe TP, Alderborn A, Anderson RD, et al. Transcriptional activation of the human papillomavirus -16 p97 promoter by an 88-nucleotide enhancer containing distinct cell-dependent and AP-1 responsive modules. *The New Biologist* 1990;2:450-463.
65. Mack D, Lamins LA. Keratinocyte-specific transcription factor, KRF-1 interacts with AP-1 to activate human papillomavirus type 18 expression in human squamous cells. *Proc. Natl. Acad. Sci. USA* 1991;88:9102-9106.

66. Gloss B, Bernard HU, Seedorf K, et al. The upstream regulatory region of human papillomavirus -16 contains an E2 protein-independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. *EMBO J.* 1987;6:3735-3743.
67. Meijer CJLM, van den Brule AJC, Snijders PJF, Helmerhorst T, Kenemans P, Walboomers JMM. Detection of human papillomavirus in cervical scrapes by the polymerase chain reaction in relation to cytology: possible implications for cervical cancer screening. In: Munoz N, Bosch FX, Shah KV, Meheus A, eds. *The epidemiology of human papillomavirus and cervical cancer.* Oxford: Oxford University Press, 1992.
68. Walboomers JMM, Melkert PWJ, van den Brule AJC, Snijders PJF, Meijer CJLM. The polymerase chain reaction for screening in diagnostic cytopathology of the cervix. In: Herrington CS, McGee OO, eds. . Oxford: IRL Press, 1992.
69. van den Brule AJC, Snijders PJF, Gordijn RLJ, Bleker OP, Meijer CJLM, Walboomers JMM. General primer-mediated polymerase chain reaction permits the detection of sequenced and still unsequenced human papillomavirus genotypes in cervical scrapes and carcinomas. *Int. J. Cancer* 1990;45:644-649.
70. van den Brule AJC, Claas HCJ, de Maine M, et al. Use of anticontamination primers in the polymerase chain reaction for the detection of human papillomavirus genotypes in cervical scrapes and biopsies. *J. Med. Virol.* 1989;29:20-27.
71. Resnick R, Cornelissen MTE, Wright DK, et al. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. *J. Natl. Cancer Inst.* 1990;82:1477-1484.
72. Shibata DK, Arnheim N, Martin JW. Detection of human papillomavirus in paraffin embedded tissue using polymerase chain reaction. *J. Exp. Med.* 1988;167:225-230.
73. Ting Y, Manos MM. Detection and typing of genital human papillomaviruses. *PCR protocols: A guide to methods and applications.*: Academic Press, 1990.
74. Bauer HM, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* 1991;265:472.
75. Melkert PW, Hopman E, van den Brule AJC, et al. Prevalence of HPV in cytomorphological normal cervical smears, as determined by the polymerase chain reaction, is age dependent. *Int. J. Cancer* 1993;53:919.
76. Barrasso R, De Brux J, Croissant O, et al. High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. *N. Engl. J. Med.* 1987;317:916.
77. McCance DJ, Kopan R, Fuchs E, et al. Human papillomavirus type 16 alters human epithelial cell differentiation in vitro. *Proc. Natl. Acad. Sci. USA* 1988;85:7169.
78. van den Brule AJC, Walboomers JMM, du Maine M, Kenemans P, Meijer CJLM. Difference in prevalence of human papillomavirus genotypes in cytomorphologically abnormal cervical smears is associated with a history of cervical intraepithelial neoplasia. *Int. J. Cancer* 1991;48:404-408.
79. Koutsky LA, Holmes KK, Crichtlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N. Engl. J. Med.* 1992;327:1272.
80. Franco ELF. Epidemiology of anogenital warts and cancer. In: Lorincz A, Reid R, eds. *Obstetrics and Gynecology Clinics of North America.* Philadelphia: W. B. Saunders Company, 1996 (vol 23).
81. Franco EL. Cancer causes revisited: Human papillomavirus and cervical neoplasia. *J. Natl. Cancer Inst.* 1995;87:779-780.
82. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: A worldwide perspective. International biological study on cervical (IBSCC) Study Group. *J. Natl. Cancer Inst.* 1995;87:796-802.
83. Franco EL. Summary and discussion: Association of HPV and anogenital cancer and implications for screening policy. In: Munoz N, Bosch FX, Shah KV, al e, eds. *The*

epidemiology of human papillomavirus and cervical cancer. Oxford: Oxford University Press, 1992.

84. Munoz N, Bosch FX, DeSanjose S, et al. The causal link between human papillomavirus and invasive cervical cancer: A population based case-control study in Colombia and Spain. *Int. J. Cancer* 1992;52:743-749.

85. Schneider A, Koutsky L. Natural history and epidemiological features of genital HPV infection. In: Munoz M, Bosch FX, Shah KV, Meheus A, eds. *The Epidemiology of Cervical Cancer and Human Papillomavirus*. Oxford: Oxford University Press, 1992.

86. Schiffman H. Epidemiology of cervical human papillomavirus infections. *Curr. Top. Microbiol. Immunol.* 1994;186:55-81.

87. Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. *J. Infect. Dis.* 1994;169:235-240.

88. Mosciski AB, Palefski J, Smith G, et al. Variability of human papillomavirus DNA testing in a longitudinal cohort of young women. *Obstet. Gynecol.* 1993;82:572-578.

89. Villa LL, Franco EL, Rahal P, et al. A cohort study of persistence of cervical HPV infection determined by molecular variant analysis. 14th International Papillomavirus Conference. Quebec, Canada, 1995.

90. Bauer HM, Hildesheim A, Schiffman MH, et al. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. *Sex Transm. Dis.* 1993;20:274-278.

91. Barbosa MS, Lowy DR, Schiller JT. Papillomavirus polypeptides E6 and E7 are zinc-binding proteins. *J. Virol.* 1989;63:1404-1407.

92. Rawls J, Pusztai R, Green M. *J. Virol.* 1990;64:6121-6129.

93. Phelps WC, Yee CL, Munger K, Howley PM. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of Adenovirus E1A. *Cell* 1988;53:539-547.

94. Smotkin D, Wettstein FO. Transcription of human papillomavirus type 16 early genes in a cervical cancer and a cancer derived cell line and identification of E7 protein. *Proc. Natl. Acad. Sci. USA* 1987;83:4680-4684.

95. Dyson N, Howley P, Munger K, Harlow E. The human papillomavirus 16-E7 is able to bind to the retinoblastoma gene product. *Science* 1989;243:934.

96. Munger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J.* 1989;8:4099.

97. Barbosa MS, Edmonds C, Fischer Cea. The region of the HPV E7 oncoprotein homologous to adenovirus E1a and SV40 large T antigen contains separate domains for Rb binding and casein kinase II phosphorylation. *EMBO J.* 1990;9:153-160.

98. Imai Y, Matsushima Y, Takashi S, Terada M. Purification and characterization of human papillomavirus type 16 E7 protein with preferential binding capacity to the underphosphorylated form of retinoblastoma gene product. *J. Virol.* 1991;65:4966-4972.

99. Edmonds C, Vousden KH. A point mutational analysis of human papillomavirus type 16 E7 protein. *J. Virol.* 1989;63:2650-2656.

100. Buchkovich K, Duffy LA, Harlow E. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. *Cell* 1989;58:1097-1105.

101. Shirodkar S, Ewens M, DeCaprio JA, et al. The transcription factor E2F interacts with the retinoblastoma product and a p107-cyclin A complex in a cell cycle-regulated manner. *Cell* 1992;68:157-166.

102. Laiho M, DeCaprio JA, Ludlow JW, Livingstone DM, Massague J. Growth inhibition by TGF-beta linked to suppression of retinoblastoma protein phosphorylation. *Cell* 1990;62:175-185.

103. Arroyo M, Bagchi S, Raychaudhuri P. Association of the human papillomavirus type 16 E7 protein with the S-phase-specific E2F-cyclin A complex. *Mol. Cell Biol.* 1993;13:6537-6546.

104. Gage JR, Meyers C, Wettstein FO. The E7 proteins of the nononcogenic human papillomavirus type 6b (HPV-6b) and of the oncogenic HPV-16 differ in retinoblastoma protein binding and other properties. *J. Virol.* 1990;64:723.
105. Munger K, Yee CL, Phelps WC. Biochemical and biological differences between E7 oncoproteins of the high and low risk HPV types are determined by aminoterminal sequences. *J. Virol.* 1991;65:3943.
106. Hawley-Nelson P, Vousden KH, Hubbert NL, Schiller JT. HPV 16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. *EMBO J.* 1989;8:3905.
107. Grossman SR, Mora R, Lamins LA. Intracellular localization and DNA-binding properties of human papillomavirus type 18 E6 protein expressed with a baculovirus vector. *J. Virol.* 1989;63:366-374.
108. Weiness BA, Levine AJ, Howley PM. Association of human papillomavirus type 16 and 18 E6 proteins with p53. *Science* 1990;248:76-79.
109. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990;63:1129-36.
110. Diller L, Kassel J, Nelson CE, et al. p53 function as a cell cycle control protein in osteosarcomas. *Mol. Cell. Biol.* 1993;10:5772-5781.
111. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild type p53. *Cell* 1992;70:923-935.
112. Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM. Wild type p53 reestablishes cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell* 1992;70:937-948.
113. Hartwell L. Defects in a cell cycle check point may be responsible for the genomic instability of cancer. *Cell* 1992;71:543-546.
114. Lane DP. p53, guardian of the genome. *Nature* 1992;358:15-16.
115. Crook T, Tidy JA, Vousden KH. Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans activation. *Cell* 1991;67:547-556.
116. Medcalf EA, Milner J. Targeting and degradation of p53 by E6 of human papillomavirus type 16 is preferential for the 1620+ p53 conformation. *Oncogene* 1993;8:2847-2851.
117. Huibregtse JM, Scheffner M, Howley P. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of HPV E6 oncoproteins with p53. *Mol. Cell Biol.* 1993;13:775-784.
118. Scheffner MK, Munger J, Byrne J, Howley P. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. *Proceedings of the National Academy of Sciences USA* 1991;88:5523.
119. Kessis TD, Slebos RJ, Nelson WG, et al. Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. *Proc. Natl. Acad. Sci. USA* 1993;90:3988.
120. Park DJ, Wilczynski SP, Paquette RL, Miller CW, Koeffler HP. p53 mutations in HPV-negative cervical carcinoma. *Oncogene* 1994;9:205-10.
121. Conrad M, Bubb VJ, Schlegel R. The HPV-6 and HPV-16 E5 proteins are membrane associated proteins which associate with the 16kD pore-forming protein. *J. Virol.* 1993;67:6170.
122. Conrad M, Goldstein D, Andresson T, et al. The E5 protein of HPV-6, but not HPV-16, associates efficiently with cellular growth factor receptors. *Virology* 1994;200:796.
123. Pim D, Collins M, Banks L. Human papillomavirus type 16 E5 gene stimulates the transforming activity of the epidermal growth factor receptor. *Oncogene* 1992;7:27.

124. Storey A, Greenfield I, Whitbeck A, et al. Lack of immortalizing activity of a human papillomavirus type 16 variant with a mutation in the E2 gene isolated from normal human cervical keratinocytes. *Oncogene* 1992;7:459.
125. Matsukura T, Koi S, Sugase M. Both episomal and integrated forms of human papillomavirus type 16 are involved in invasive cervical cancers. *Virology* 1989;172:63.
126. May M, Dong XP, Beyer-Finkler E, et al. The E6/E7 promoter of extrachromosomal HPV 16 DNA in cervical cancers escapes from cellular repression by mutation of target sequences for YY1. *EMBO J.* 1994;13:1460.
127. Dong XP, Stubenrauch F, Beyer-Finkler E, et al. Prevalence of deletions of YY1-binding sites in episomal HPV 16 DNA from cervical cancers. *Int. J. Cancer* 1994;58:803.
128. Ho L, Chan SY, Chow V, et al. Sequence variants of human papillomavirus type 16 in clinical samples permit verification and extension of epidemiological studies and construction of a phylogenetic tree. *J. Clin. Microbiol.* 1991;29:1765.
129. Karre K, Ljunggren HG, Piontek G, Kiesling R. Selective rejection of H-2 deficient lymphoma variants suggest alternative immune defense strategy. *Nature* 1986;319:675-678.
130. Boog CJP, Kast WM, Timmers M, Boes HT, de Waal J, Melief CJM. Abolition of specific immune response defect by immunization with dendritic cells. *Nature* 1985;318:59-62.
131. Altmann A, Jochmuskudielka I, Frank R, et al. Definition of immunogenic determinants of the human papillomavirus type-16 nucleoprotein-e7. *Eur J Cancer* 1992;28:326-333.
132. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T-cells. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol* 1986;136:2348-2357.
133. Mossmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol.* 1989;7:145-173.
134. Fiorentino DF, Bond MW, Mosmann TM. Two types of mouse helper T-cells. II. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 1989;170:2081-2095.
135. Fargeas C, Wu CY, Nakajima T, Cox D, Nutman T, Delespesse G. Differential effect of transforming growth factor beta on the synthesis of Th1 and Th2 like lymphokines by human lymphocytes. *Eur. J. Immunol.* 1992;22:2173-2176.
136. Weaver CT, Hawrylywski CM, Unanue ER. T helper subsets require the expression of distinct co-stimulatory signals by antigen-presenting cells. *Proc. Natl. Acad. Sci. USA* 1988;85:8181-8185.
137. Unanue ER. Cellular studies on antigen presentation by class II MHC molecules. *Curr. Opin Immunol.* 1992;4:63-69.
138. Bjorkman PJ, Saper MA, Samraoui B, Bennet WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 1987;329:506-512.
139. Robb RJ, Munck J, Smith KA. T-cell growth factor receptors: Quantitation, specificity and biological relevance. *J. Exp. Med.* 1981;154:1455-1474.
140. Smith KA. Interleukin-2. *Ann. Rev. Immunol.* 1984;2:319-333.
141. Hackett CJ, Yewdell JW, Bennik JR, Syssocka M. Class II MHC restricted T-cell determinants processed from either endosomes or the cytosol with similar requirements for host protein transport but different kinetics of production. *J. Immunol.* 1991;146:2944-2951.
142. Peitsch MC, Tschopp J. Assembly of macromolecular pores by immune defense systems. *Curr. Opin Cell Biol.* 1991;3:710-716.
143. Dupuis M, Schaerer E, Krause KH, Tschopp J. The calcium-binding protein calreticulin is a major constituent of lytic granules in cytolytic T lymphocytes. *J. Exp. Med.* 1993;177:1-7.

144. Chatila TA, Geha RS, Arnaout MA. Constitutive and stimulus-induced phosphorylation of CD11 CD18 leukocyte adhesion molecules. *J. Cell. Biol.* 1989;109:3435-3444.
145. Dustin ML, Springer TA. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989;341:619-624.
146. Kupfer A, Singer SJ. Cell biology of cytotoxic and helper T cell functions: immunofluorescence microscopic studies of single cells and cell couples. *Ann. Rev. Immunol.* 1989;7:309-337.
147. Yanelli JR, Sullivan JA, Mandell G, Englehard VH. Reorientation and fusion of cytotoxic T lymphocytes granules after interaction with target cells as determined by high resolution cinemicrography. *J. Immunol.* 1986;136:377-382.
148. Podack ER, Kupfer A. T-cell effector functions: mechanisms for delivery of cytotoxicity and help. *Ann. Rev. Cell. Biol.* 1991;7:479-504.
149. Peters PJ, Geuze HJ, van der Donk HJ, Borst J. A new model for lethal hit delivery by cytotoxic T lymphocytes. *Immunol. Today* 1990;11:28-32.
150. Old LJ. Tumor necrosis factor (TNF). *Science* 1985;230:630-632.
151. Trowsdale J, Ragoussis J, Campbell RD. Map of the Human MHC. *Immunol Today* 1991;12:443-446.
152. Figueroa F, Gunther E, Klein J. MHC polymorphism predating speciation. *Nature* 1988;335:265-267.
153. Hughes AL, Nei M. Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. *Proc. Natl. Acad. Sci. USA* 1988;86:958-962.
154. McConnel TJ, Talbot WS, McIndoe RA, Wakeland EK. The origin of MHC class II gene polymorphism within the genus *Mus*. *Nature* 1988;332:651-654.
155. Lawlor DA, Ward FE, Ennis PD, Jackson AP, Parham P. HLA-A and B polymorphisms predate the divergence of humans and chimpanzees. *Nature* 1988;335:268-271.
156. Gustafsson K, Wiman K, Emmoth E, et al. Mutations and selection in the generation of class II histocompatibility antigen polymorphism. *EMBO J.* 1984;3:1655-1661.
157. Bjorkman PJ, Parham P. Structure, function and diversity of class I major histocompatibility complex molecules. *Ann. Rev. Biochem.* 1990;59:253-288.
158. Pease LR, Horton RM, Pullen JK, Cai Z. Structure and diversity of class I antigen presenting molecules in the mouse. *Crit. Rev. Immunol.* 1991;11:1-32.
159. Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ. The detailed distribution of HLA-A, B, C antigens in normal human organs. *Transpl.* 1984;38:287-291.
160. Garrett TPJ, Saper MA, Bjorkman PJ, Strominger JL, Wiley DC. Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature* 1989;342:692-696.
161. Madden DR, Gorga JC, Strominger JL, Wiley DC. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature* 1991;353:321-325.
162. Robinson MA, Kindt TJ. *Fundamental Immunology*. In: Paul WE, ed. New York: Raven Press, 1989.
163. Gorga JC. Structural analysis of class II major histocompatibility complex proteins. *Crit. Rev. Immunol.* 1992;11:305-335.
164. Brown JH, Jardetzky TS, Gorga JC, et al. 3 Dimensional structure of the human class II histocompatibility antigen HLA DR1. *Nature* 1993;364:33-39.
165. Stern LJ, Brown JH, Jardetzky TS, et al. Crystal structure of the human class II MHC protein HLA DR1 complexed with an influenza virus peptide. *Nature* 1994;368:215-221.
166. Matsumura M, Fremont DH, Peterson PA, Wilson IA. Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science* 1992;257:927-934.

167. Sette A, Buus S, Colon S, Smith JA, Miles C, Grey HM. Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. *Nature* 1987;328:395-399.
168. Jardetzky TS, Gorga JC, Busch R, Rothbard J, Strominger JL, Wiley DC. Peptide binding to HLA-DR1: a peptide with most residues substituted to alanine retains MHC binding. *EMBO J.* 1990;9:1797.
169. Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG. Allele specific peptide motifs revealed by sequencing of self peptides eluted from MHC molecules. *Nature* 1991;351:290-296.
170. Hammer J, Valsasnini P, Tolba K, et al. Promiscuous and allele specific anchors in HLA-DR-binding peptides. *Cell* 1993;74:197-203.
171. Boehncke WH, Takeshita T, Pendleton CD, et al. The importance of dominant negative effects of amino acid side chain substitution in peptide-MHC molecule interactions and T cell recognition. *J. Immunol.* 1993;150:331-341.
172. Sette A, Sidney J, Gaetta FCA, et al. MHC class II molecules bind indiscriminately self and non self peptide homologs: effect on the immunogenicity of non-self peptides. *Int. Immunol.* 1993;5:631.
173. Hammer J, Belunis C, Bolin D, et al. High affinity binding of short peptides to major histocompatibility complex class II molecules by anchor combinations. *Proc. Natl. Acad. Sci. USA* 1994;91:4456-4460.
174. Chicz RM, Urban RG, Gorga JC, et al. Specificity and promiscuity among naturally bound peptides bound to HLA-DR alleles. *J. Exp. Med.* 1993;178:27-47.
175. Rudensky AY, Preston-Hurlburt P, Al-Ramadi BK, Rothbard J, Janeway CA. Truncation variants of peptides isolated from MHC class II molecules suggest sequence motifs. *Nature* 1992;359:429.
176. Morrison LA, Lukacher AE, Braciale VL, Fan DP, Braciale TJ. Differences in antigen presentation to MHC class I and class II restricted influenza virus-specific cytolytic T lymphocyte clones. *J. Exp. Med.* 1986;163:903-921.
177. Braciale TJ, Morrison LA, Sweetser MT, Sambrook J, Gething MJ, Braciale VL. Antigen presentation pathways to class I and class II MHC-restricted T lymphocytes. *Immunol. Rev.* 1987;98:95-114.
178. Tanaka K, Tamura T, Yoshimura T, Ichihara A. Proteasomes: protein and gene structures. *New Biol.* 1992;4:173-187.
179. Monaco JJ. A molecular model of MHC class-I restricted antigen presentation. *Immunol. Today* 1992;13:173-178.
180. Driscoll J, Brown MG, Finley D, Monaco JJ. MHC-linked LMP gene products specifically alter peptidase activities of the proteasome. *Nature* 1993;365:262-264.
181. Gagzynska M, Rock KL, Goldberg AL. Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature* 1993;365:264-267.
182. Tsomides TJ, Eisen HN. Antigenic structures recognized by cytotoxic T lymphocytes. *J. Biol. Chem.* 1991;266:3357-3360.
183. Braciale TJ. Antigen processing for presentation by MHC class I molecules. *Curr. Opin. Immunol.* 1992;4:59-62.
184. Yewdell JW, Bennink JR. Brefeldin A specifically inhibits presentation of protein antigens to cytotoxic T lymphocytes. *Science* 1989;244:1072-1075.
185. Cox JH, Yewdell JW, Eisenlohr LC, Johnson PR, Bennink JR. Antigen presentation requires transport of MHC class I molecules from the endoplasmic reticulum. *Science* 1990;247:715-718.
186. Gething MJ, Sambrook J. Protein folding in the cell. *Nature* 1992;355:33-45.
187. Degen E, Cohen-Doyle MF, Williams DB. Efficient dissociation of the p88 chaperone from major histocompatibility complex class I molecules requires both β 2-microglobulin and peptide. *J. Exp. Med.* 1992;175:1653-1661.
188. Cerundolo V, Alexander J, Anderson K, et al. Presentation of viral antigen controlled by a gene in the major histocompatibility complex. *Nature* 1990;345:449-452.

189. Kelly A, Powis SH, Kerr LA, et al. Assembly and function of the two ABC transporter proteins encoded in the human histocompatibility complex. *Nature* 1992;355:641-644.
190. Brodsky FM. *Trends Cell Biol.* 1992;2:109-115.
191. Long EO. Antigen processing for presentation to CD4+ T cells. *New Biol.* 1992;4:274-282.
192. Jones PP, Murphy DB, Hewgill D, McDevitt HO. Detection of a common polypeptide chain in I-A and I-E subregion immunoprecipitates. *Mol. Immunol.* 1979;16:51-60.
193. Teyton L, Peterson PA. *Trend Cell Biol.* 1992;2:52-56.
194. Roche PA, P. C. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 1990;345:615-618.
195. Teyton L, O'Sullivan D, Dickson PW, et al. Invariant chain distinguishes between the exogenous and endogenous antigen presentation pathways. *Nature* 1990;348:39-44.
196. Roche PA, Teletski CL, Karp DR, Pinet V, Bakke O, Long EO. Stable surface expression of invariant chain prevents peptide presentation by HLA-DR. *EMBO J.* 1992;11:2841-2847.
197. Neefjes JJ, Ploegh HL. Intracellular transport of MHC class II molecules. *Immunol. Today* 1992;13:179-184.
198. Blum JS, Cresswell P. Role of intracellular proteases in the processing and the transport of class II HLA antigens. *Proc. Natl. Acad. Sci. USA* 1988;85:3975-3979.
199. Maric MA, Taylor MD, Blum JS. Endosomal aspartic proteinases are required for invariant-chain processing. *Proc. Natl. Acad. Sci. USA* 1994;91:2171-2175.
200. Riberdy JM, Cresswell P. The antigen-processing mutant T2 suggests a role for MHC-linked genes in class II antigen processing. *J. Immunol.* 1992;148:2586-2590.
201. Cresswell P. Assembly, transport and function of MHC class II molecules. *Annu. Rev. Immunol.* 1994;12:259-293.
202. Kropshofer H, Vogt AB, Stern LJ, Hammerling GJ. Self-release of CLIP in peptide loading of HLA-DR molecules. *Science* 1995;270:1357-1359.
203. Sloan VS, Camerson P, Porter G, et al. Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature* 1995;375:802-806.
204. Denzin LK, Cresswell P. HLA-DM induces CLIP dissociation from MHC class II ab dimers and facilitates peptide loading. *Cell* 1995;82:155-165.
205. Sherman MA, Weber DA, Jensen PE. DM enhances peptide binding to class II MHC by release of invariant-chain derived peptide. *Immunity* 1995;3:197-205.
206. Bennet K, Levine T, Ellis JS, et al. Antigen processing for presentation by class II major histocompatibility complex requires cleavage by cathepsin E. *Eur. J. Immunol.* 1992;22:1519-1524.
207. van Noort JM, Boon J, van der Drift ACM, Wagenaar JPA, Boots AHM, Boog CJP. Antigen processing by endosomal proteases determines which sites of sperm-whale myoglobin are eventually recognized by T-cells. *Eur. J. Immunol.* 1991;21:1981-1996.
208. Bidwell JL, Bidwell EA, Savage DA, Middleton D, Klouda PT, Bradley BA. A DNA-RFLP typing system that positively identifies serologically well-defined and ill-defined HLA DR and DQ alleles, including DRw10. *Transplantation* 1988;45:640.
209. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of β globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 1985;230:1350.
210. Saiki RK, Chang CA, Levenson CH, Warren TC, Boehm CD, Kazazian HH. Diagnosis of sickle cell anemia and beta thalassemia with enzymatically amplified DNA and nonradioactive allele-specific oligonucleotide probes. *N. Engl. J. Med.* 1988;319:537-541.
211. Saiki RK, Walsh PS, Levenson CH, et al. Genetic analysis of amplified DNA with immobilised sequence-specific oligonucleotide probes. *Proc. Natl. Acad. Sci. USA* 1989;86:6230-6234.

212. Gentilomi G, Musiani M, Zerbini M, Gallinella G, Gibellini D, La Placa M. A hybrido-immunocytochemical assay for the in-situ detection of cytomegalovirus DNA using digoxigenin-labeled probes. *J. Immunol. Methods* 1989;125:177.
213. Kimura A, Dong RP, Harada H, Sasazuki T. DNA typing of HLA class II genes in B-lymphoblastoid cell lines homozygous for HLA. *Tissue Antigens* 1992;40:5.
214. Tiercy JM, Gorski J, Jeannet M, Mach B. Identification and distribution of three serologically undetected alleles of HLA-DR by oligonucleotide DNA typing analysis. *Proc. Natl. Acad. Sci. USA* 1988;85:198.
215. Eliaou JF, Humbert M, Balaguer P, et al. A method of class II typing using nonradioactive oligonucleotides. *Tissue Antigens* 1989;33:475.
216. Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for HLA-DNA typing. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA* 1991. Oxford: Oxford University Press, 1992 (vol 1).
217. Erlich H, Bugawan T, Begovich AB, et al. HLA-DR, DQ and DP typing using PCR amplification and immobilised probes. *Eur. J. Immunogenet.* 1991;18:33-35.
218. Erlich HA, Bugawan TL. HLA class II gene polymorphism: DNA typing, evolution, and relationship to disease susceptibility. In: Erlich HA, ed. *PCR Technology: Principles and Application for DNA Amplification*. New York: Stockton Press, 1989.
219. Olerup O. HLA class II typing by digestion of PCR-amplified DNA with allele specific restriction endonucleases will fail to unequivocally identify the genotypes of many homozygous and heterozygous individuals. *Tissue Antigens* 1990;36:83-87.
220. Newton CR, Graham A, Heptinsall LE, et al. Analysis of any point mutation in DNA: the amplification refractory mutation system (ARMS). *Nucleic Acids Res.* 1989;17:2503.
221. Olerup O, Zetterquist H. HLA DR typing by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-reipient matching in cadaveric transplantation. *Tissue Antigens* 1992;39:225.
222. Bein G, Glaser R, Kirchner H. Rapid HLA-DRB1 genotyping by nested PCR amplification. *Tissue Antigens* 1992;39:68.
223. Krausa P, Brywka M, Savage D, et al. Genetic polymorphism within HLA-A*02: significant allelic variation revealed in different population. *Tissue Antigens* 1995;45:223.
224. Clay TM, Bidwell JL, Howard MR, Bradley BA. PCR-finger-printing for selection of HLA matched unrelated marrow donors. Collaborating centers in the IMUST study. *Lancet* 1991;337:1049.
225. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single stranded polymorphisms. *Proc. Natl. Acad. Sci. USA* 1989;86:2766.
226. Santamaria P, Boyce JM, Lindstrom AL, Barbosa JJ, Faras AJ, Rich SS. HLA class II "typing": direct sequencing of DRB, DQB, and DQA genes. *Hum. Immunol.* 1992;33:69.
227. Rozemuller EH, Bouwens AG, Bast BE, Tilanus MG. Assignment of HLA-DPB alleles by computerized matching based upon sequence data. *Hum. Immunol.* 1993;37:207.
228. Meyers C, Frattini MG, Hudson JB, Laminis LA. Biosynthesis of human papillomavirus type 31b from a continuous cell line upon epithelial differentiation. *Science* 1992;257:971-973.
229. Vardy DA, Baadsgaard O, Hansen ER, Lisby S, Vejlsgaard GL. The cellular immune response to human papillomavirus infection. *Int. J. Dermatol.* 1990;29:603-610.
230. Jenson AB, Kurman RJ, Lancaster WD. Tissue effects of and host response to human papillomavirus infection. *Dermatol Clin* 1991;9:203-209.
231. Roche JK, Crum CP. Local immune response and the uterine cervix : implications for cancer associated viruses. *Cancer Immunol. Immunother.* 1991;33:203-209.

232. Ghosh A, Smith NK, Stacey SN, et al. Serological responses to HPV 16 in cervical dysplasia and neoplasia. Correlation of antibodies to E6 with cervical cancer. *Int J Cancer* 1993;53:591-596.
233. Cason J, Best JM. Antibody responses to human papillomavirus type 16 infections. *Rev. Med. Virol.* 1991;1:201-209.
234. Lin YL, Borrenstein LA, Selvakumar R, Ahmed R, Wettstein FO. Progression from papilloma to carcinoma is accompanied by changes in antibody response to papillomavirus proteins. *J. Virol.* 1993;67:382-389.
235. Jochmus-Kudielka J, Scheider A, Braun R, Kimmong R, Koldovsky U, Schneeweis KE, et al. Antibodies against the human papillomavirus type 16 early proteins in human sera : correlation of anti E7 reactivity with cervical cancer. *J Natl Cancer Inst* 1989;81:1698-1704.
236. Reid TMS, Fraser NG, Kernohan IR. Generalized warts and immune deficiency. *Br J Dermatol* 1976;95:559-564.
237. Morrison WL. Cell mediated immunity in patients with warts. *Br J Dermatol* 1975;93:553-556.
238. Lagar M, Icenogle JP, Marsella R, et al. Genital papillomavirus infection and cervical dysplasia - opportunistic complication of HIV infection. *Int. J. Cancer* 1992;50:45-48.
239. Halpert R, Frutcher RG, Sedlis A, Butt K, Boyce JG, Sillman FH. Human papillomavirus and lower genital neoplasia in renal transplant patients. *Obstet. Gynecol.* 1986;68:251-258.
240. Alloub MI, Barr BBB, MacClaren K, Smith IW, Bunney MH, Smart GE. Human papillomavirus infection and cervical intraepithelial neoplasia in women with renal allografts. *Br. Med. J.* 1989;298:153-156.
241. Hawthorn RJS, Murdorch JB, Maclean AB, Mackie RM. Langerhans cells and subtypes of human papillomavirus in cervical intraepithelial neoplasia. *Br Med J* 1988;297:643-646.
242. Tay SK, Jenkins D, Maddox P, Campion M, Singer A. Subpopulations of Langerhans cells in cervical neoplasia. *Br. J. Obstet. Gynaecol.* 1987;94:10-15.
243. Malejczuk J, Majewski S, Jablonska S, Ragozinski TT, Orth G. Abrogated NK cell lysis of HPV 16 bearing keratinocytes in patients with precancerous and cancerous HPV induced anogenital lesions. *Int. J. Cancer* 1988;43:209-214.
244. McKenzie J, King A, Hare J, Fulford T, Wilson B, Stanley M. Immunocytochemical characterization of large granular lymphocytes in normal cervix and HPV associated disease. *J Pathol* 1991;165:75-80.
245. Benton C, Shahidullah H, Hunter JAA. Human papillomavirus in the immunosuppressed. *Papillomavirus Rep.* 1992;3:23-26.
246. Levine BJM, Hardwill BT, Trapp TO, Crawford R, Bollinger C, Griffin DE. Antibody mediated clearance of alphavirus infection from neurones. *Science* 1991;254:856.
247. Neefjes JJ, Momburg F. Cell biology of antigen presentation. *Curr. Opinion Immunol.* 1993;5:27-34.
248. Terasaki PI, Perdue ST, Mickey MR. HLA frequencies in cancer: A second study. In: Mulvihill JJ MR, Fraumeni JF Jr, ed. . New York: Raven Press, 1977.
249. Takasugi M, Terasaki PI, Henderson B, Mickey MR, Menck H, Thompson RW. HLA antigens in solid tumours. *Cancer Res.* 1973;33:648-650.
250. Tarpley JL, Chretien PB, Rogentine N, Twomey PL, Dellon AL. Histocompatibility antigens and solid malignant neoplasms. *Arch. Surg.* 1975;110:269-271.
251. Twomey PL, Rogentine GN, Chretien PB. Lymphocyte function and HLA antigen frequency in gynecologic squamous cancer. *Int. Surg.* 1974;59:468-472.
252. Hammond MG, Appadoo B, Brain P. HLA and cancer in South African Indians. *Tissue Antigens* 1979;14:296-302.

253. Hammond MG, Appadoo B, Brain P. HLA and cancer in South African Negroes. *Tissue Antigens* 1977;9:1-7.
254. Koenig UD, Muller N, Schneweis KE. Herpes simplex type 2 antibodies and HLA B12 in cervical cancer. *Lancet* 1976;ii:857.
255. Wank R, Thomssen C. High risk of squamous cell carcinoma of the cervix for women with HLA-DQW3. *Nature* 1991;352:723-725.
256. Wank R, Schendel DJ, Thomssen C. HLA antigens and cervical carcinoma. *Nature* 1992;356:22-23.
257. Wank R, Meulen JT, Luande J, Eberhardt HC, Pawlita M. Cervical intraepithelial neoplasia, cervical carcinoma, and risk for patients with HLA-DQB1*0602, *0301, *0303 alleles. *The Lancet* 1993;341:1215.
258. Klitz W, Aldrich CL, Fildes N, Horning SJ, Begovich AB. Localization of predisposition to Hodgkin's disease in the HLA class II region. *Amer. J. Hum. Genet.* 1994;54:497-505.
259. Panza N, Del Vecchio L, Maio M, et al. Strong association between an HLA DR antigen and thyroid carcinoma. *Tissue Antigens* 1982;20:155-158.
260. Czanecki DB, Lewis A, Nicholson I, Tait B. Multiple non-melanoma skin cancer associated with HLA DR7 in Southern Australia. *Cancer* 1991;68:439-440.
261. Lee JE, Reveille JD, Ross MI, Platsoucas CD. HLA-DQB1*0301 association with increased cutaneous melanoma risk. *Int. J. Cancer* 1994;59:510-513.
262. Lu S, Day NE, Degos L, et al. Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region. *Nature* 1990;346:470-471.
263. Ghosh P, Amaya M, Mellins E, Wiley DC. The structure of an intermediate in class II MHC maturation - clip bound to HLA DR3. *Nature* 1995;378:457-462.
264. Rammensee HG, Friede T, Stevanovic S. MHC ligands and peptide motifs: first listing. *Immunogenetics* 1995;41:178.
265. Kreiger JJ, Karr RW, Grey HM, et al. Single amino acid changes in DR and antigen define residues critical for peptide-MHC binding and T cell recognition. *J. Immunol.* 1991;146:2331.
266. Sidney J, Oseroff C, Southwood S, et al. DRB1*0301 molecules recognize a structural motif distinct from the one recognized by most DRB1 alleles. *J. Immunol.* 1992;149:2634.
267. Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG. Pool sequencing of natural HLA-DR, DQ, and DP ligands reveals detailed peptide motifs, constraints of processing, and general rules. *Immunogenetics* 1994;39:230-242.
268. Hammer J, Takacs B, Sinigaglia F. Identification of a motif for HLA-DR1 binding peptides using M13 display libraries. *J. Exp. Med.* 1992;176:1007.
269. Verreck FAW, van de Poel A, Termijtlen. A, et al. Identification of an HLA- DQ2 peptide binding motif and HLA- DPw3-bound self peptide by pool sequencing. *Eur. J. Immunol.* 1994;24:375-379.
270. Freide T, Gnau V, Jung G, Keilholz W, Stevanovic S, Rammensee H. Natural ligand motifs of closely related HLA-DR4 molecules predict features of rheumatoid arthritis associated peptides. *Biochim. Biophys. Acta* 1996;1316:85-101.
271. O'Sullivan D, Arrhenius T, Sidney J, et al. On the interaction of promiscuous antigenic peptides with different DR alleles. *J. Immunol.* 1991;147:2664-2669.
272. Vogt AB, Kropshofer H, Kalbacher H, et al. Ligand motifs of HLA DRB5*0101 and DRB1*1501 molecules delineated from self peptides. *J. Immunol.* 1994;24:375-379.
273. Wucherpfenning KW, Sette A, Southwood S, et al. Structural requirements for binding an immunodominant myelin basic protein peptide to DR2 isotypes and for its recognition by human T cell clones. *J. Exp. Med.* 1994;279:290.
274. Hammer J, Bono E, Gallazi F, Belunis C, Nagy Z, Sinigaglia F. Precise prediction of major histocompatibility complex class II-peptide interaction based on peptide side chain scanning. *J. Exp. Med.* 1994;180:2353-2358.

275. Marshall KW, Wilson KJ, Liang J, Woods A, Zaller D, Rothbard JB. Prediction of peptide affinity to HLA DRB1*0401. *J. Immunol.* 1995;154:5927-5933.
276. Hammer J, Sinigaglia F. HLA class II peptide binding specificity and autoimmunity. *Adv. Immunol.* 1997;66:67-100.
277. Anderson M, Brown C, Buckley C, et al. Current views on cervical intra-epithelial neoplasia. *J. Clin. Pathol.* 1991;44:969-978.
278. Seedorf K, Krammer G, Durst M, Suhai S, Rowekamp WG. Human papillomavirus type 16 DNA sequence. *Virology* 1985;145:181-185.
279. Cole ST, Danos O. Nucleotide sequence and comparative analysis of the human papillomavirus type 18 genome. Phylogeny of papillomaviruses and repeated structure of the E6 and E7 gene products. *J. Mol. Biol.* 1987;193:599-608.
280. Goldsborough MD, DiSilvestre D, Lorincz AT. Nucleotide sequence of human papillomavirus type 31: a cervical neoplasia-associated virus. *Virology* 1989;171:306-311.
281. Cole ST, Streeck RE. Genome organization and nucleotide sequence of human papillomavirus type 33, which is associated with cervical cancer. *J. Virol.* 1986;58:991-995.
282. Sambrook J, Fritsch T, Maniatis T. *Molecular cloning: A laboratory manual.* . Cold Spring Harbor, NY: Cold Spring Harbor Press., 1989.
283. Eiken HG, Odland E, Boman H, Skjelkvale L, Engebretsen LF, Apold J. Application of natural and amplification created restriction sites for the diagnosis of PKU mutations. *Nucleic Acids Res.* 1991;19:1427-1430.
284. Olerup O, Aldener A, Fogdell A. HLA-DQB1 and DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. *Tissue Antigens* 1993;41:119-134.
285. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;239:487-491.
286. Begovich AB, McClure GR, Suraj VC, et al. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J. Immunol.* 1992;148:249-258.
287. Doherty DG, Vaughan RW, Donaldson PT, Mowat AP. HLA DQA, DQB, and DRB genotyping by oligonucleotide analysis: distribution of alleles and haplotypes in British Caucasoids. *Hum. Immunol.* 1993;34:53-63.
288. Breslow NE, Day NE. *The analysis of case control studies. Statistical methods in cancer research.* Lyon: IARC, 1980 (vol 1).
289. Armitage P. *Statistical methods in medical research.* . Oxford: Blackwell, 1971.
290. Sidney J, Oseroff C, del Guercio M, et al. Definition of a DQ3.1-specific binding motif. *J. Immunol.* 1994;152:4516-4525.
291. Johansen BH, Vartdal F, Eriksen JA, Thorsby E, Sollid LM. Identification of a putative motif for binding of peptides to HLA-DQ2. *Int. Immunol.* 1995;8:177-182.
292. Johansen B, Jensen T, Thorpe C, Vartdal F, Thorsby E, Sollid L. Both the alpha and beta chain determine the binding specificity of HLA-DQ2. *Hum. Immunol.* 1996;47:19.
293. Chiczy R, Lane W, Robinson R, Trucco M, Strominger J, Gorga J. Self peptides bound to the type 1 diabetes associated class II MHC molecules HLA-DQ1 and HLA DQ8. *Int. Immunol.* 1994;6:1639.
294. Kwok W, Nepom GT, Raymond FC. HLA-DQ polymorphisms are highly selective for peptide binding interactions. *J. Immunol.* 1995;155:2468-2476.
295. Kwok W, Domeier E, Raymond F, Byers P, Nepom G. Allele-specific motifs characterize HLA -DQ interactions with a diabetes associated peptide derived from glutamic acid decarboxylase. *J. Immunol.* 1996;156:2171.
296. Vartdal F, Johansen BH, Friede T, et al. The peptide binding motif of the disease associated HLA-DQ (alpha 1*0501, beta 1*0201) molecule. *Eur. J. Immunol.* 1996;26:2764-2772.

297. Godkin A, Freide T, Davenport M, et al. Use of eluted peptide sequence data to identify the binding characteristics of peptides to the insulin-dependent diabetes susceptibility allele HLA-DQ8(DQ3.2). *Int. Immunol.* 1997;9:905-911.
298. Edman P. Method for determination of the amino acid sequence in peptides. *Acta Chem. Scand.* 1950;4:283.
299. Edman P, Begg G. A protein sequenator. *Eur. J. Biochem.* 1967;1:80.
300. Odunsi KO, Ganesan TS. The roles of the human major histocompatibility complex and human papillomavirus infection in cervical intra-epithelial neoplasia and cervical cancer. *Clinical Oncology* 1997;9:4-13.
301. Zur Hausen H. Viruses in human cancers. *Science* 1991;254:1167-1173.
302. Mehal WZ, Lo YMD, Herrington CS, et al. Human papillomavirus infection plays an important role in determining the HLA associated risk of cervical carcinogenesis. *J. Clin. Path.* 1994;47:1077-1081.
303. Vandenvelde C, deFoor M, vanBeers D. HLA DQB1*03 and cervical intraepithelial neoplasia grades I-III. *The Lancet* 1993;341:442-444.
304. Bonagura VR, O'Riley ME, Abramson AL, Steinberg BM. Recurrent respiratory papillomatosis (RRP): Enriched HLA DQW3 phenotype and decreased class I MHC expression. 12th International papillomavirus conference., 1993.
305. Han R, Breitburd F, Marche PN, Orth G. Linkage of regression and malignant conversion of rabbit viral papillomas to MHC class II genes. *Nature* 1992;356:66-68.
306. Apple RJ, Erlich HA, Klitz W, Manos MM, Becker TM, Wheeler CM. HLA DR-DQ associations with cervical carcinoma show papillomavirus-type specificity. *Nat Genet* 1994;6:157-62.
307. Chen LP, Thomas EK, Hu SL, Hellstrom I, Hellstrom KE. Human papillomavirus type 16 nucleoprotein E7 is a tumor rejection antigen. *Proc. Natl. Acad. Sci. USA* 1991;88:110-114.
308. Odunsi K, Terry G, Ho L, Bell J, Cuzick J, Ganesan TS. Association between HLA DQB1*03 and cervical intraepithelial neoplasia. *Mol. Med.* 1995;1:161-171.
309. Apple RJ, Becker TM, Wheeler CM, Erlich HA. Comparison of human leukocyte antigen DR-DQ disease associations found with cervical dysplasia and invasive cervical carcinoma. *J Natl Cancer Inst* 1995;87:427-36.
310. Morrison EA, Ho GY, Vermund SH, et al. Human papillomavirus infection and other risk factors for cervical neoplasia: a case control study. *Int. J. Cancer* 1991;49:6-13.
311. Buus S, Sette A, Colon SM, Grey HM. Autologous peptides constitutively occupy the antigen binding site on Ia. *Science* 1988;242:1045-1047.
312. Huczko EL, Bodnar WM, Benjamin D, et al. Characteristics of endogenous peptides eluted from the class I MHC molecule HLA-B7 determined by mass spectrometry and computer modelling. *J. Immunol.* 1993;151:2572-2587.
313. Fremont DH, Matsumara M, Stura EA, Peterson PA, Wilson IA. Crystal structures of two viral peptides in complex with murine MHC class I H-2Kb. *Science* 1992;257:919-927.
314. Madden DR, Gorga JC, Strominger JL, Wiley DC. The 3 dimensional structure of HLA B27 at 2.1Å resolution suggests a general mechanism for tight peptide binding to MHC. *Cell* 1992;70:1035-1048.
315. Hill CM, Hayball JD, Allison AA, Rothbard JB. Conformational and structural characteristics of peptides binding to HLA DR molecules. *J. Immunol.* 1991;147:189.
316. O'Sullivan D, Sidney J, Apella E, et al. Characterization of the specificity of peptide binding to four DR haplotypes. *J. Immunol.* 1990;145:1799.
317. Hill C, Liu A, Marshall K, et al. Exploration of requirements for peptide binding to HLA DRB1*0101 and DRB1*0401. *J. Immunol.* 1994;152:2890.
318. Marshall KW, Liu AF, Canales J, et al. Role of polymorphic residues in HLA-DR molecules in allele-specific binding of peptide ligands. *J. Immunol.* 1994;152:4516-4525.

319. Guo HC, Jardetzky TS, Garret TPJ, Lane WS, Strominger JL, Wiley DC. Different length peptides bind to HLA-Aw68 similarly at their ends but bulge out in the middle. *Nature* 1992;360:364.
320. Rothbard JB, Marshall K, Wilson KJ, Fugger L, Zaller D. Prediction of peptide affinity to HLA DRB1*0401. *Int. Arch. Allergy Immunol.* 1994;105:1-7.
321. Van de Wal Y, Kooy YMC, Drijfhout JW, Amons R, Konning F. Peptide binding characteristics of the coeliac disease-associated DQ(A1*0501,B1*0201) molecule. *Immunogenetics* 1996;44:246-253.
322. Kwok WW, Domeier ME, Johnson ML, Nepom GT, Koelle DM. HLA-DQB1 codon 57 is critical for peptide binding and recognition. *J. Exp. Med.* 1996;183:1253-1258.
323. Raddrizzani L, Sturniolo T, Guenot J, et al. Different modes of peptide interaction enable HLA-DQ and HLA-DR molecules to bind diverse peptide repertoires. *J. Immunol.* 1997;159:703-711.
324. Sette A, Vitiello A, Rehman B, et al. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *J. Immunol.* 1994;153:5586-5592.
325. Odunsi K, Terry G, Ho L, Bell J, Cuzick J, Ganesan TS. Susceptibility to human papillomavirus associated cervical intra-epithelial neoplasia is determined by specific HLA DR-DQ alleles. *Int. J. Cancer* 1996;67:595-602.
326. Helland A, Borresen AL, Kaern J, Ronningen KS, Thorsby E. HLA antigens and cervical carcinoma. *Nature* 1992;356:23.
327. Helland A, Borresen AL, Kristensen G, Ronningen KS. DQA1 and DQB1 genes in patients with squamous cell carcinoma of the cervix: relationship to human papillomavirus infection and prognosis. *Cancer Epidem., Biomarkers & Prevention.* 1994;3:479-486.
328. Gregoire L, Lawrence WD, Kukuruga D, Eisenbrey AB, Lancaster WD. Association between HLA-DQB1 alleles and risk for cervical cancer in African-American women. *Int J Cancer* 1994;57:504-7.
329. Nawa A, Nishiyama Y, Kobayashi T, et al. Association of HLA DQB1*03 with cervical cancer in Japanese women aged 35 years and younger. *Cancer* 1995;75:518-521.
330. Amar A, Battat S, Anteby SO, Brautbar C, Reubinoff BE. Invasive squamous cell carcinoma of the cervix: is HLA DQ a disease marker in Jewish patients? *Eur. J. Immunogenetics* 1993;20:327-333.
331. Sastre-Garau X, Loste M, Vincent-Salomon A, et al. Decreased frequency of HLA-DRB1*13 alleles in Frenchwomen with HPV-positive carcinoma of the cervix. *Int. J. Cancer* 1996;69:159-164.
332. Allen M, Kalantari M, Ylitalo N, et al. HLA DQ-DR haplotype and susceptibility to cervical carcinoma: indications of increased risk for development of cervical carcinoma in individuals infected with HPV 18. *Tissue Antigens* 1996;48:32-37.
333. Glew SS, Duggan KM, Ghosh AK, et al. Lack of association of HLA polymorphisms with human papillomavirus-related cervical cancer. *Hum Immunol* 1993;37:157-64.
334. Ellis JR, Keating PJ, Baird J, et al. The association of an HPV 16 oncogene variant with HLA B7 has implications for vaccine design in cervical cancer. *Nature Medicine* 1995;1:464-470.
335. David ALM, Taylor G, Gokhale D, Aplin JD, Seif MW, Tindall VR. HLA DQB1*03 and cervical intraepithelial neoplasia type III. *The Lancet* 1993;340:52.
336. Sanjeevi CB, Hjelmstrom P, Hallmans G, et al. Different HLA DR-DQ haplotypes are associated with cervical intraepithelial neoplasia among human papillomavirus type-16 seropositive and seronegative Swedish women. *Int. J. Cancer* 1996;68:409-414.
337. Helland A, Olsen AO, Gjoen K, et al. An increased risk of cervical intra-epithelial neoplasia grade II-III among human papillomavirus positive patients with the HLA DQA1*0102-DQB1*0602 haplotype: a population based case-control study of Norwegian women. *Int. J. Cancer* 1998;76:19-24.

338. Orth G, Favre M, Breidburd F, et al. Epidermodysplasia verruciformis. A model to understand the role of papillomaviruses in human cancer. In: Essex Mea, ed. Viruses in naturally occurring cancers. Cold Spring Harbor, NY.: Cold Spring Harbor Laboratory Press, 1980.
339. Lanchbury JSS, Sakkas LI, March SGE, Bodmer JG, Welsh KI, Panayi GS. HLA-DQ beta 3.1 allele is a determinant of susceptibility to DR4-associated rheumatoid arthritis. *Hum. Immunol.* 1989;26:59-71.
340. Orrechia G, Perfetti L, Malagoli P, Borghini F, Kipervarg Y. Vitiligo is associated with a significant increase in HLA-A30, Cw6 and DQw3 and a decrease in C4Q0 in northern Italian patients. *Dermatology* 1992;185:123-127.
341. Ahmed AR, Foster S, Zaltas M, et al. Association of DQw7 (DQB1*0301) with ocular cicatricial pemphigoid. *Proc. Nat. Acad. Sci.* 1991;88:11579-11582.
342. Khalil I, Lepage V, Douay C, et al. HLA DQB1*0301 allele is involved in the susceptibility to erythema multiforme. *J. Invest. Dermatol.* 1991:697-700.
343. Uno H, Kawano K, Matsuoka H, Tsuda K. HLA and adult T cell leukemia: HLA-linked genes controlling susceptibility to human T cell leukemia virus type 1. *Clin. exp. Immunol.* 1988;71:211-216.
344. Lee JE, Lowy AM, Thompson WA, et al. Association of gastric adenocarcinoma with HLA class II gene DQB1*0301. *Gastroenterology* 1996;111:426-432.
345. Ishikura H, Ishikawa N, Aizawa M. Differential expression of HLA class II antigens in the thymus - relative paucity of HLA-DQ antigens in the thymic medulla. *Transplantation* 1987;44:314-317.
346. Nishimura Y, Sasazuki T. Suppressor T cells control the HLA-linked immunological low responsiveness to streptococcal antigen in man. *Nature* 1983;301:67.
347. Sasazuki T, Ohta N, Kaneoka R, Kojima S. Association between an HLA haplotype and low responsiveness to schistosomal worm antigen in man. *J. Exp. Med.* 1980;152:314.
348. Ottenhoff THM, Walford C, Nishimura Y, Reddy NBB, Sasazuki T. HLA DQ molecules and the control of mycobacterium leprae specific T cell non-responsiveness in lepromatous leprosy patients. *Eur. J. Immunol.* 1990;20:2347.
349. Sasazuki T, Kohno Y, Iwamoto I, Tanimura M, Naito S. Association between an HLA haplotype and low responsiveness to tetanus toxoid in man. *Nature* 1978;272:359.
350. Hatae K, Kimura A, Okubo Rea. Genetic control of non-responsiveness to hepatitis B virus vaccine by an extended HLA haplotype. *Eur. J. Immunol.* 1992;22:1899-1905.
351. Salagne P, Covit J, Bloom BR. Immunological suppression by human CD8+ T cells is receptor dependent and HLA DQ restricted. *Proc. Natl. Acad. Sci. USA* 1991;88:2598-2602.
352. Sasazuki T, Kikuchi K, Hirayama S, Matsushita S, Ohta N, Nishimura Y. HLA linked immune suppression in humans. *Immunol* 1989;S2:21-25.
353. Hu FY, Asano Y, Sano K, Inoue T, Furutani-Seiki M, Tada T. Establishment of stable CD8+ suppressor T cell clones and the analysis of their suppressive function. *J. Immunol. Methods* 1992;152:123-134.
354. Fairchild RL, Palmer E, Moorhead JW. Production of DNP-specific/class I MHC restricted suppressor molecules is linked to the expression of T cell receptor α - and β - chain genes. *J. Immunol.* 1993;150:67-77.
355. Cone RE, Weischedel AK, Urbanski M, Kristie J. Specific antigen binding proteins secreted by an antigen specific T cell hybrid. *Mol. Immunol.* 1992;29:689-696.
356. Sun D, Qin Y, Chluba J, Epplen J, T., Werkerle H. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. *Nature* 1988;332:843-845.
357. Pauels HG, Austrup F, Becker C, Scmitt E, Rude E, Kolsch E. Lymphokine profile and activation of pattern of two unrelated antigen- or idiotype-specific T suppressor cell clones. *Eur. J. Immunol.* 1992;22:1961-1966.

358. Altman DM, Sansom D, Marsh SGE. What is the basis of HLA-DQ associations with autoimmune disease? *Immunol. Today* 1991;12:267-270.
359. Gillis S. T-cell derived lymphokines. In: Paul WE, ed. *Fundamental Immunology*. New York: Raven Press, 1989.
360. Fossum B, Gedde-Dahl III T, Hansen T, Eriksen JA, Thorsby E, Gaudernack G. Overlapping epitopes encompassing a point mutation (12Gly to Arg) in p21 ras can be recognized by HLA-DR, -DP and -DQ restricted T cells. *Eur. J. Immunol.* 1993;23:2687-2691.
361. Glew SS, Duggan-Keen M, Cabrera T, Stern PL. HLA Class II antigen expression in human papillomavirus-associated cervical cancer. *Cancer Res.* 1992;52:4009-4016.
362. Hirayama K, Matsushita S, Kikuchi I, Iuchi M, Ohta N, Sasazuki T. HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. *Nature* 1987;327:426-430.
363. Paliakasis K, Routsias J, Petratos K, Ouzounis C, Kokknidis M, Papadopoulos GK. Modelling of the structure of the human histocompatibility molecules HLA DQ based on the published structure of HLA -DR1. 13th International Immunology and Diabetes Workshop, May 25-28, 1994. Montvillagerne, France., 1994.
364. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate T cell clones specific for myelin basic protein. *Cell* 1995;80:695-705.
365. Konig R, Huang YL, Germain RN. MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. *Nature* 1992;356:799-801.
366. Springer TA. Traffic signals for leukocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;76:301-312.
367. Hynes RO. Integrins: versatility, modulation and signalling in cell adhesion. *Cell* 1992;69:11-25.
368. Janossy G, Thomas JA, Bollum FJ. The human thymic microenvironment: an immunohistochemical study. *J. Immunol.* 1980;125:202-212.
369. Wade WF, Davoust J, Salamero J, Pascale A, Watts TH, Cambier JC. Structural compartmentalization of MHC class II signalling function. *Immunol. Today* 1993;14:539-546.
370. Morzycka-Wroblewska E, Harwood JI, Smith JR, Kagnoff MS. Structure and evolution of the promoter regions of the DQA genes. *Immunogenetics* 1993;37:364-372.
371. Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Cullen A, Kurman RJ. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. *Am. J. Obstet. Gynecol.* 1995;172:946-954.
372. Wright TC, Sun XW, Koulos J. Comparison of management algorithms for the evaluation of women with low grade cytologic abnormalities. *Obstet. Gynecol.* 1995;85:202-210.
373. Kinney WK, Manos MM, Hurley LB, Ransley JE. Where's the high-grade cervical neoplasia? The importance of minimally abnormal papanicolaou diagnosis. *Obstet. Gynecol.* 1998;91:973-976.
374. Wu T, Guarnier FG, Staveley-O'Carroll KF, et al. Engineering an intracellular pathway for major histocompatibility complex class II presentation of antigens. *Proc. Natl. Acad. Sci. USA* 1995;92:11671-11675.
375. Lin KY, Guarnieri FG, Staveley-O'Carroll KF, et al. Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. *Cancer Research* 1996;56:21-26.
376. Cormier JN, Salgaller ML, Prevette T, et al. Enhancement of cellular immunity in melanoma patients immunized with a peptide from MART-1/Melan A. *Cancer J. Sci. Am.* 1997;3:37-44.

377. Salgaller ML, Marincola FM, Cormier JN, Rosenberg SA. Immunization against epitopes in the human melanoma antigen gp100 following patient immunization with synthetic peptides. *Cancer Res.* 1996;56:4749-4757.
378. Meuer SC, Hodgdon JC, Cooper DA, et al. Human cytotoxic T cell clones directed at autologous virus-transformed targets : Further evidence for linkage of genetic restriction to T4 and T8 surface glycoprotein. 1983.
379. Celis E, Ou D, Otvos J. Recognition of hepatitis B surface antigen by human T lymphocytes. Proliferative and cytotoxic responses to a major antigenic determinant defined by synthetic peptides. *J. Immunol* 1987;140:1808-1815.
380. Yasukawa M, Zarlring JM. Human cytotoxic T cell clones directed against herpes simplex virus infected cells. Lysis restricted by class II DR antigens. *J. Immunol* 1984;133:422-427.
381. Golumbek PT, Lazenby AJ, Levisky HI, et al. Treatment of established renal cancer by tumor cells engineered to secrete interleukin-4. *Science* 1991;254:713-716.
382. Dranoff G, Jaffe E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony stimulating factor stimulates potent, specific, and long lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* 1993;90:3539-3543.
383. Topalian SL, Rivoltini L, Mancini M, Ng J, Hartzman RJ, Rosenberg SA. Melanoma specific CD4+ T lymphocytes recognize human melanoma antigens processed and presented by Epstein-Barr virus- transformed B cells. *Int. J. Cancer* 1994;58:69-79.
384. Topalian SL, Rivoltini L, Mancini M, et al. Human CD4+ T cells recognize a shared melanoma-associated antigen encoded by the tyrosine gene. *Proc. Natl. Acad. Sci. USA* 1994;91:9461-9465.
385. van den Burg SH, Visseren MJ, Brandt RM, Kast WM, Melief CJ. Immunogenicity of peptides bound to MHC class I molecules depends on the MHC-peptide complex stability. *J. Immunol.* 1996;156:3308-3314.
386. Chen L, Mizuno MT, Singhal MC, et al. Induction of cytotoxic T lymphocytes specific for a syngeneic tumor expressing the E6 oncoprotein of human papillomavirus type 16. *J. Immunol.* 1992;148:2617-21.
387. Meneguizzi G, Cerni C, Kieny MP, Lathe R. Immunization against human papillomavirus type 16 tumor cells with recombinant vaccinia viruses expressing E6 and E7. *Virology* 1991;181:62-69.
388. Feltkamp MCW, Smits HL, Vierboom MPM, et al. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16 transformed cells. *Eur J Immunol* 1993;23:2242-2249.
389. Kast WM, Brandt RMP, Sidney J, et al. Role of HLA-A motifs in identification of potential CTL epitopes in human papillomavirus type 16 E6 and E7 proteins. *J. Immunol.* 1994;3904-3912.
390. Rensing ME, Sette A, Brandt RM, et al. Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201 binding peptides. *J. Immunol.* 1995;154:5934-5943.
391. Storey A, Thomas M, Kalita A, et al. Role of a p53 polymorphism in the development of human papilloma virus associated cancer. *Nature.* 1998;393:229-234.
392. Sercarz EE, Lehmann PV, Ametani A, Benichou F, Miller A, Moudgil K. Dominance and crypticity of T-cell antigenic determinants. *Annu. Rev. Immunol.* 1993;11:729-766.

APPENDIX I: FULL HLA DR-DQ RESULTS ON PATIENTS WITH CIN

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-----------|--------|------|-------------|------------|------------|------------|------|------------------|------------------|---------------|----------------------|-------------------------|-------------------------|
| Sample No | CIN | HPV | DQB1 | DRB1 | DRB3 | DRB4 | DRB5 | Inferred Haplo.1 | Inferred Haplo.2 | CephDO-DRB1.1 | CephDO-DRB1.2 | DQ-DRB1-DRB345.1 | DQ-DRB1-DRB345.2 |
| 1 | MP1001 | CIN1 | 33I | 0303/0601 | 0301/1301 | 0301/0202 | 0 | 0 | 0 | 0303/1301 | 0601/0301 | 0301/1301/B3*0301 | 0601/0301/B3*0202 |
| 2 | MP108 | CIN1 | 31H | 0201/0302 | 0301/0404 | *0101 | DR53 | 0 | 0 | 0201/0301 | 0302/0404 | 0201/0301/B3*0101 | 0302/0604/B4*0101(2) |
| 3 | MP1122 | CIN3 | 18H | 0303/05031 | 1301/1401 | *0101 | 0 | 0 | 0 | 0303/1301 | 0503/1101 | 0303/1301/B3*0101 | 0503/1101/B3*0101 |
| 4 | MP1139 | CIN1 | Negative | 0201/0603 | 0301/1301 | *0101 | 0 | 0 | 0 | 0201/0301 | 0603/1301 | 0201/0301/B3*0101 | 0603/1301/B3*0101 |
| 5 | MP1152 | CIN1 | Negative | 0201/0601 | 0301/1501 | *0201 | 0 | *0101 | 0 | 0201/0301 | 0601/1501 | 0201/0301/B3*0201 | 0601/1501/B5*0101 |
| 6 | MP1171 | CIN1 | 33H | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 7 | MP1221 | CIN1 | 16I | 0401/0602 | 0401/1502 | 0 | DR53 | *0101 | 0 | 0401/0401** | 0602/1502** | 0401/0401/B4*0101(2) | 0602/1502/B5*0101 |
| 8 | MP1230 | CIN3 | 16H | 0301/0601 | 1501/1201 | *0202 | 0 | *0201/2 | 0 | 0601/1501** | 0301/1201 | 0601/1501/B5*0201(2) | 0301/1201/B3*0202 |
| 9 | MP1236 | CIN3 | 16H | 0201/0501 | 0301/DR10 | *0101 | 0 | 0 | 0 | 0201/0301 | 0501/1001 | 0201/0301/B3*0101 | 0501/1001/0 |
| 10 | MP1237 | CIN1 | 16L 31H 33H | 0301/0605 | 0401/DR7 | 0 | DR53 | 0 | 0301/0401 | 0605/0701(2)* | 0301/0401/B4*0101(2) | 0605/0701(2)/B4*0101(2) | 0605/0701(2)/B4*0101(2) |
| 11 | MP137 | CIN3 | 16H | 0301/0301 | 0801/0801 | 0 | 0 | 0301/0801 | 0301/0801 | 0301/0801** | 0301/0801** | 0301/0801/0 | 0301/0801/0 |
| 12 | MP1425 | CIN3 | 16H | 0302/0601 | 1501/0401 | 0 | DR53 | 0201/2 | 0 | 0601/1501** | 0302/0401 | 0601/1501/B5*0201(2) | 0302/0401/B4*0101(2) |
| 13 | MP143 | CIN3 | 16H 18H | 0201/0302 | 0301/0404 | *0202 | DR53 | 0 | 0 | 0201/0301 | 0302/0404 | 0201/0301/B3*0202 | 0302/0404/D4*0101(2) |
| 14 | MP1446 | CIN3 | 18H | 0201/0602 | 0301/1502 | *0202 | 0 | *0102 | 0 | 0201/0301 | 0602/1502** | 0201/0301/B3*0202 | 0602/1502/B5*0102 |
| 15 | MP145 | CIN1 | 18H | 0301/0603 | 1101/0401 | *0202 | DR53 | 0 | 0 | 0603/1101** | 0301/0401 | 0603/1101/B3*0202 | 0301/0401/B4*0101(2) |
| 16 | MP1460 | CIN1 | 18I | 0201/0604 | 0301/1302 | *0101 | 0 | 0 | 0 | 0201/0301 | 0604/1302 | 0201/0301/B3*0101 | 0604/1302/B3*0101 |
| 17 | MP1490 | CIN3 | 31H | 0201/0601 | 0301/1501 | *0101 | 0 | 0201/2 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0201(2)* |
| 18 | MP1505 | CIN1 | Negative | 0201/0402 | 0801/0301 | *0101 | 0 | 0 | 0 | 0402/0801** | 0201/0301 | 0402/0801/0 | 0201/0301/B3*0101 |
| 19 | MP1629 | CIN3 | Negative | 0201/0601 | 0301/1501 | *0101 | 0 | *0201/2 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0201(2)* |
| 20 | MP166 | CIN3 | 16H | 0301/0303 | 0103/DR9 | 0 | 0 | 0 | 0 | 0301/0103** | 0303/0901** | 0301/0103/0 | 0303/0901/0 |
| 21 | MP167 | CIN3 | 16H 31H 33H | 0201/0302 | 0301/0402 | *0202 | DR53 | 0 | 0 | 0201/0301 | 0302/0402 | 0201/0301/B3*0202 | 0302/0402/B4*0101(2) |
| 22 | MP200 | CIN3 | 18H 31H | 0301/0302 | 0401/0404 | 0 | DR53 | 0 | 0 | 0301/0401 | 0302/0404 | 0301/0401/B4*0101(2) | 0302/0404/B4*0101(2) |
| 23 | MP201 | CIN1 | 31H | 0201/0302 | 0404/DR7 | 0 | DR53 | 0 | 0 | 0302/0404 | 0201/0701(2) | 0302/0404/B4*0101(2) | 0202/0701(2)/B4*0101(2) |
| 24 | MP230 | CIN1 | 16H | 0301/0601 | 0407/DR7 | 0 | DR53 | 0 | 0 | 0301/0407* | 0601/0701(2)* | 0301/0407/B4*0101(2) | 0601/0701(2)/B4*0101(2) |
| 25 | MP247 | CIN3 | 16H | 0302/05031 | 0401/1401 | *0101 | 0 | 0 | 0 | 0302/0401 | 0503/1101 | 0302/0401/0 | 0503/1101/B3*0101 |
| 26 | MP253 | CIN1 | Negative | 0301/0301 | 1101/1101 | *0202 | 0 | 1101/0301 | 1101/0301 | 1101/0301 | 1101/0301 | 1101/0301/B3*0202 | 1101/0301/B3*0202 |
| 27 | MP255 | CIN3 | Negative | 0302/0301 | 0403/1303 | *0202 | DR53 | 0 | 0 | 0302/0403 | 0301/1303 | 0302/0403/B4*0101(2) | 0301/1303/B3*0202 |
| 28 | MP262 | CIN1 | Negative | 0303/0604 | 1301/1301 | *0101/0301 | DR53 | 0 | 0303/1301 | 0604/1301 | 0303/1301 | 0303/1301/B3*0301 | 0604/1301/B3*0101 |
| 29 | MP306 | CIN1 | 33H | 0201/0501 | DR7/0101 | 0 | DR53 | 0 | 0 | 0201/0701(2) | 0501/0101 | 0201/0701(2)/B4*0101(2) | 0501/0101/0 |
| 30 | MP320 | CIN3 | 16I | 0201/0301 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0201/0301 | 0301/0401 | 0201/0301/B3*0101 | 0301/0401/B4*0101(2) |
| 31 | MP352 | CIN1 | Negative | 0301/0201 | 0301/0402 | *0101 | DR53 | 0 | 0 | 0201/0301 | 0301/0402** | 0201/0301/B3*0101 | 0301/0402/B4*0101(2) |
| 32 | MP401 | CIN1 | 31H | 0301/0402 | 0801/0801 | 0 | 0 | 0301/0801 | 0402/0801 | 0301/0801** | 0402/0801** | 0301/0801/0 | 0402/0801/0 |
| 33 | MP446 | CIN3 | 31H | 0201/0601 | 0301/1502 | *0101 | 0 | *0102 | 0 | 0201/0301 | 0601/1502 | 0201/0301/B3*0101 | 0601/1502/B5*0102 |
| 34 | MP449 | CIN3 | 31H | 0301/0501 | 0401/0802 | *0202 | DR53 | 0 | 0 | 0301/0401 | 0501/0802* | 0301/0401/B4*0101(2) | 0501/0802/B3*0202* |
| 35 | MP53 | CIN1 | Negative | 0501/0601 | 1501/0103 | 0 | 0 | *0201/2 | 0 | 0601/1501** | 0501/0103 | 0601/1501/B5*0201(2) | 0501/0103/0 |
| 36 | MP567 | CIN1 | Negative | 0301/0601 | 1501/0401 | 0 | DR53 | *0201/2 | 0 | 0601/1501** | 0301/0401 | 0601/1501/B5*0201(2) | 0301/0401/B4*0101 |
| 37 | MP569 | CIN3 | 16H | 0201/0201 | 0301/0301 | *0202 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 38 | MP584 | CIN1 | 16H | 0302/0201 | 0301/0403 | *0101 | DR53 | 0 | 0 | 0201/0301 | 0302/0403 | 0201/0301/B3*0101 | 0302/0403/B4*0101(2) |
| 39 | MP621 | CIN3 | 16H | 0201/0301 | 0406/0301 | 0 | DR53 | 0 | 0 | 0301/0406 | 0201/0301 | 0301/0406/B4*0101(2) | 0201/0301/0 |
| 40 | MP63 | CIN3 | 16H 18H | 0301/0302 | DR9/0103 | 0 | DR53 | 0 | 0 | 0302/0901* | 0301/0103** | 0302/0901/B4*0101(2) | 0301/0103/0 |
| 41 | MP633 | CIN3 | 16H | 0301/0601 | 1301/08031 | *0202 | 0 | 0 | 0 | 0301/1301** | 0601/08031* | 0301/1301/B3*0202 | 0601/08031/0 |
| 42 | MP652 | CIN1 | 16H | 0303/0303 | DR7/DR9 | 0 | DR53 | 0 | 0303/0701(2) | 0303/0701(2) | 0303/0901** | 0303/0701(2)/B4*0101(2) | 0303/0901/B4*0101(2) |
| 43 | MP686 | CIN3 | 16H | 0301/0303 | 0103/DR9 | 0 | DR53 | 0 | 0 | 0301/0103** | 0303/0901** | 0301/0103/0 | 0303/0901/B4*0101(2) |
| 44 | MP706 | CIN1 | 33H | 0201/0302 | 0301/0406 | *0101 | DR53 | 0 | 0 | 0201/0301 | 0302/0406* | 0201/0301/B3*0101 | 0302/0406/B4*0101(2) |
| 45 | MP742 | CIN1 | 18H | 0301/0501 | 0101/0402 | 0 | DR53 | 0 | 0 | 0501/0101 | 0301/0402** | 0501/0101/0 | 0301/0402/B4*0101(2) |
| 46 | MP743 | CIN3 | Negative | 0201/0302 | 0404/0301 | *0101 | DR53 | 0 | 0 | 0302/0404 | 0201/0301 | 0302/0404/B4*0101(2) | 0201/0301/B3*0101 |
| 47 | MP752 | CIN3 | Negative | 0302/0602 | 0404/0801 | *0201/0202 | DR53 | 0 | 0 | 0302/0404 | 0602/0801* | 0302/0404/B4*0101(2) | 0602/0801/B3*0201* |
| 48 | MP765 | CIN1 | Negative | 0502/0604 | 1501/1302 | *0301 | 0 | *0201/2 | 0 | 0502/1501 | 0604/1302 | 0502/1501/B5*0201(2) | 0604/1302/B3*0301 |
| 49 | MP810 | CIN1 | Negative | 05032/0501 | 1401/0101 | *0101 | 0 | 0 | 0 | 05032/1401 | 0501/0101 | 05032/1401/B3*0101 | 0501/0101/0 |
| 50 | MP899 | CIN1 | 33H | 0201/0201 | 0301/0301 | *0101 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 51 | MP904 | CIN1 | 31H | 0301/0301 | 1101/1104 | *0202 | 0 | 0301/1101 | 0301/1104 | 0301/1101 | 0301/1104 | 0301/1101/B3*0202 | 0301/1104/B3*0202 |
| 52 | MP922 | CIN3 | 18L 33I | 0302/0602 | 1501/DR7 | 0 | DR53 | *0201/2 | 0 | 0602/1501 | 0602/0701(2)** | 0602/1501/B5*0201(2) | 0602/0701(2)/B4*0101(2) |
| 53 | MP945 | CIN1 | Negative | 0601/0603 | 1501/1301 | *0202 | 0 | *0101 | 0 | 0601/1501** | 0603/1301 | 0601/1501/B5*0101 | 0603/1301/B3*0202 |
| 54 | MP969 | CIN1 | Negative | 0201/0301 | 0301/1104 | *0202 | 0 | 0 | 0 | 0201/0301 | 0301/1104 | 0201/0301/B3*0202 | 0301/1104/B3*0202 |
| 55 | MP995 | CIN3 | 16H | 0201/0302 | 0301/0404 | *0101 | DR53 | 0 | 0 | 0201/0301 | 0302/0404 | 0201/0301/B3*0101 | 0302/0404/B4*0101(2) |
| 56 | NT106 | CIN3 | 16H | 0301/0601 | 0401/1502 | 0 | DR53 | *0102 | 0 | 0301/0401 | 0601/1502 | 0301/0401/B4*0101(2) | 0601/1502/B5*0102 |
| 57 | NT111 | CIN3 | 31L | 0201/0602 | 0301/1501 | *0101 | 0 | *0201/2 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0101 | 0602/1501/B5*0201(2) |
| 58 | NT112 | CIN1 | 31L 33L | 0301/0402 | 0401/1301 | *0101 | DR53 | 0 | 0 | 0301/0401 | 0402/1301* | 0301/0401/B4*0101(2) | 0402/1301/B3*0101 |
| 59 | NT115 | CIN3 | Negative | 0302/0201 | 0401/0301 | *0101 | DR53 | 0 | 0 | 0302/0401 | 0201/0301 | 0302/0401/B4*0101(2) | 0201/0301/B3*0101 |
| 60 | NT116 | CIN3 | 16H 18L 33H | 0302/0301 | 0401/0406 | 0 | DR53 | 0 | 0 | 0301/0401 | 0302/0406* | 0301/0401/B4*0101(2) | 0302/0406/B4*0101(2) |
| 61 | NT117 | CIN3 | 31H | 0201/0301 | 0301/1304 | *0202 | 0 | 0 | 0 | 0201/0301 | 0301/1304** | 0201/0301/B3*0202 | 0301/1304/B3*0202 |
| 62 | NT126 | CIN1 | 16L | 0201/0201 | 0401/0301 | *0202 | DR53 | 0 | 0201/0401 | 0201/0401* | 0201/0301 | 0201/0401/B4*0101(2) | 0201/0301/B3*0202 |
| 63 | NT128 | CIN3 | 18H | 0301/0602 | 1501/0802 | *0202 | 0 | *0201/2 | 0 | 0602/1501 | 0301/0802** | 0602/1501/B5*0201(2) | 0301/0802/B3*0202* |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-----|-------|------|--------------|------------|------------|------------|------|---------|--------------|--------------|----------------|----------------------|-------------------------|-------------------------|
| 65 | NT129 | CIN3 | 31H | 0301/0302 | 0401/0406 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0302/0406 | 0301/0401/B4*0101(2) | 0302/0406/B4*0101(2) |
| 66 | NT131 | CIN3 | 16H18131H33H | 0201/0301 | 0404/DR7 | 0 | DR53 | 0 | 0 | 0 | 0301/0404* | 0201/0701(2) | 0301/0404/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 67 | NT133 | CIN3 | 16H 33H | 0302/0302 | 0401/DR7 | 0 | DR53 | 0 | 0302/0401 | 0302/0701(2) | 0302/0401 | 0302/0701(2)** | 0302/0401/B4*0101(2) | 0302/0701(2)/B4*0101(2) |
| 68 | NT137 | CIN3 | 16H | 0301/0201 | 0301/0401 | 0 | DR53 | 0 | 0 | 0 | 0201/0301 | 0301/0401 | 0201/0301/0* | 0301/0401/B4*0101(2) |
| 69 | NT143 | CIN3 | 16H 31I | 0301/05032 | 1401/0103 | *0202 | 0 | 0 | 0 | 0 | 05032/1401 | 0301/0103** | 05032/1401/B3*0202 | 0301/0103/0 |
| 70 | NT144 | CIN3 | Negative | 0201/0301 | 1101/0301 | *0202 | 0 | 0 | 0 | 0 | 0301/1101 | 0201/0301 | 0301/1101/B3*0202 | 0201/0301/B3*0202 |
| 71 | NT160 | CIN3 | Negative | 0201/0302 | 0401/0301 | *0101 | DR53 | 0 | 0 | 0 | 0301/0401 | 0201/0301 | 0302/0401/B4*0101(2) | 0201/0301/B3*0101 |
| 72 | NT161 | CIN3 | 16H | 0302/0402 | 1303/0803 | *0202 | 0 | 0 | 0 | 0 | 0302/1303* | 0402/0803* | 0302/1303/B3*0202 | 0402/0803/0 |
| 73 | NT28 | CIN3 | 16L 33H | 0501/0502 | 0101/1501 | 0 | 0 | *0201/2 | 0 | 0 | 0501/0101 | 0502/1501 | 0501/0101/0 | 0502/1501/B5*0201(2) |
| 74 | NT29 | CIN3 | 16131L33H | 0402/0502 | 1601/0801 | 0 | 0 | *0201/2 | 0 | 0 | 0502/1601 | 0402/0801** | 0502/1601/B5*0201(2) | 0402/0801/0 |
| 75 | NT30 | CIN3 | 16I | 0201/0602 | 0301/1501 | *0101 | 0 | *0201/2 | 0 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0101 | 0602/1501/B5*0201(2) |
| 76 | NT33 | CIN3 | 16H | 0301/0301 | 0401/1101 | *0202 | DR53 | 0 | 0301/0401 | 0301/1101 | 0301/0401 | 0301/1101 | 0301/0401/B4*0101(2) | 0301/1101/B3*0202 |
| 77 | NT36 | CIN3 | 16L 18L | 0201/0603 | 1301/0301 | *0101 | 0 | 0 | 0 | 0 | 0603/1301 | 0201/0301 | 0603/1301/B3*0101 | 0201/0301/B3*0101 |
| 78 | NT41 | CIN3 | 16H | 0604/0605 | 1302/0101 | *0301 | 0 | 0 | 0 | 0 | 0604/1302 | 0605/0101* | 0604/1302/B3*0301 | 0605/0101/0 |
| 79 | NT42 | CIN3 | 16H | 0302/0302 | 1406/1406 | *0101 | 0 | 0 | 0302/1406 | 0302/1406 | 0302/1406* | 0302/1406/B3*0101 | 0302/1406/B3*0101 | 0302/1406/B3*0101 |
| 80 | NT46 | CIN3 | 16H 31H | 0301/0501 | 0101/1201 | *0202 | 0 | 0 | 0 | 0 | 0501/0101 | 0301/1201 | 0501/0101/0 | 0301/1201/0202 |
| 81 | NT47 | CIN3 | 16H 31L | 0302/0302 | 0407/0407 | 0 | DR53 | 0 | 0302/0407 | 0302/0407 | 0302/0407 | 0302/0407/B4*0101(2) | 0302/0407/B4*0101(2) | 0302/0407/B4*0101(2) |
| 82 | NT53 | CIN3 | 16L 31H | 0401/0601 | 0401/1501 | 0 | DR53 | 0201/2 | 0 | 0 | 0401/0401** | 0601/1501** | 0401/0401/B4*0101(2) | 0601/1501/B5*0201(2) |
| 83 | NT56 | CIN3 | 16L 31H | 0301/0301 | 0401/0406 | 0 | DR53 | 0 | 0301/0406 | 0301/0401 | 0301/0406* | 0301/0401 | 0301/0406/B4*0101(2) | 0301/0401/B4*0101(2) |
| 84 | NT58 | CIN1 | Negative | 0603/0604 | 1101/1302 | *0202/0301 | 0 | 0 | 0 | 0 | 0603/1101** | 0604/1302 | 0603/1101/B3*0202 | 0604/1302/B3*0301 |
| 85 | NT62 | CIN3 | 16H | 0301/0601 | 0407/1501 | 0 | DR53 | *0101 | 0 | 0 | 0301/0407 | 0601/1501** | 0301/0407/B4*0101(2) | 0601/1501/B5*0101 |
| 86 | NT64 | CIN3 | 16H 18H | 0601/0601 | 1501/1502 | 0 | 0 | *0201/2 | 0601/1501 | 0601/1502 | 0601/1501** | 0601/1502 | 0601/1501/B5*0201(2) | 0601/1502/B5*0201(2) |
| 87 | NT67 | CIN3 | 16H | 0604/0504 | 1401/1302 | *0301/0101 | 0 | 0 | 0 | 0 | 0504/1401 | 0604/1302 | 0504/1401/B3*0101 | 0604/1302/B3*0301 |
| 88 | NT71 | CIN3 | 16H | 0201/0601 | 0301/1101 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0601/1101* | 0201/0301/B3*0202 | 0601/1101/B3*0202 |
| 89 | NT72 | CIN3 | 16L 31H | 0302/0601 | 0401/1502 | 0 | DR53 | *0102 | 0 | 0 | 0302/0401 | 0601/1502 | 0302/0401/B4*0101(2) | 0601/1502/B5*0102 |
| 90 | NT73 | CIN3 | 16H | 0401/0502 | 1602/0404 | 0 | DR53 | 0201/2 | 0 | 0 | 0502/1602** | 0401/0404* | 0502/1602/B5*0201(2) | 0401/0404/B4*0101(2) |
| 91 | NT74 | CIN3 | 16H | 0302/0201 | DR7/DR7 | 0 | DR53 | 0 | 0302/0701(2) | 0201/0701(2) | 0302/0701(2)** | 0201/0701 | 0302/0701(2)/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 92 | NT79 | CIN3 | 16H | 0302/0302 | 0403/DR7 | 0 | DR53 | 0 | 0302/0403 | 0302/0701(2) | 0302/0403 | 0302/0701(2)** | 0302/0403/B4*0101(2) | 0302/0701(2)/B4*0101(2) |
| 93 | NT80 | CIN3 | 16H | 0302/0601 | 1501/0401 | 0 | DR53 | *0101 | 0 | 0 | 0601/1501 | 0302/0401 | 0601/1501/B5*0101 | 0302/0401/B4*0101(2) |
| 94 | NT83 | CIN3 | 31H | 0302/0602 | 1502/0403 | 0 | DR53 | *0102 | 0 | 0 | 0602/1502 | 0302/0403 | 0602/1502/B5*0102 | 0302/0403/B4*0101 |
| 95 | NT86 | CIN3 | 16I | 0201/0602 | 0301/1501 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0101 | 0602/1501/B5*0101 |
| 96 | NT90 | CIN3 | 31H | 0301/0301 | 1201/0401 | *0101 | DR53 | 0 | 0301/1201 | 0301/0401 | 0301/1201 | 0301/0401 | 0301/1201/B3*0101 | 0301/0401/B4*0101(2) |
| 97 | NT96 | CIN3 | 16L | 0401/0604 | 0103/1301 | *0301 | 0 | 0 | 0 | 0 | 0401/0103* | 0604/1301 | 0401/0103/0 | 0604/1301/B3*0301 |
| 98 | W10 | CIN1 | Negative | 0201/0301 | 0301/08042 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/08042 | 0201/0301/B3*0101 | 0301/08042/0 |
| 99 | W100 | CIN3 | 16H | 05031/0604 | DR7/DR9 | 0 | DR53 | 0 | 0 | 0 | 0604/0701(2)* | 05031/0901* | 0604/0701(2)/B4*0101(2) | 05031/0901/B4*0101(2) |
| 100 | W102 | CIN3 | 31L | 05032/0604 | 1602/1301 | *0101 | 0 | *0102 | 0 | 0 | 05032/1602* | 0604/1301** | 05032/1602/B5*0102 | 0604/1301/B3*0101 |
| 101 | W104 | CIN1 | Negative | 0201/0602 | 0302/DR9 | *0101 | DR53 | 0 | 0 | 0 | 0201/0302* | 0602/0901* | 0201/0302/B3*0101 | 0602/0901/B4*0101(2) |
| 102 | W11 | CIN1 | 31H | 0301/0302 | 0103/0402 | 0 | DR53 | 0 | 0 | 0 | 0301/0103** | 0302/0402 | 0301/0103/0 | 0302/0402/B4*0101(2) |
| 103 | W111 | CIN3 | 16L | 0401/0501 | 0401/0101 | 0 | DR53 | 0 | 0 | 0 | 0401/0401** | 0501/0101 | 0401/0401/B4*0101(2) | 0501/0101/0 |
| 104 | W112 | CIN3 | 16H | 0301/0301 | 1101/1202 | *0202 | 0 | 0 | 0301/1101 | 0301/1202 | 0301/1101 | 0301/1202** | 0301/1101/B3*0202 | 0301/1202/B3*0202 |
| 105 | W12 | CIN1 | 16L | 0301/0504 | 0401/1401 | *0202 | DR53 | 0 | 0 | 0 | 0301/0401 | 0504/1401** | 0301/0401/B4*0101(2) | 0504/1401/B3*0202 |
| 106 | W120 | CIN3 | 16H | 0301/0301 | 0401/0404 | 0 | DR53 | 0 | 0301/0401 | 0301/0404 | 0301/0401 | 0301/0404* | 0301/0401/B4*0101(2) | 0301/0404/B4*0101(2) |
| 107 | W121 | CIN3 | Negative | 0502/05031 | 1401/1601 | *0202 | 0 | 0201/2 | 0 | 0 | 05031/1401 | 0502/1601 | 05031/1401/B3*0202 | 0502/1601/B5*0201(2) |
| 108 | W125 | CIN3 | Negative | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 109 | W128 | CIN3 | Negative | 0302/0302 | 1101/1101 | *0101 | 0 | 0 | 0302/1101 | 0302/1101 | 0302/1101 | 0302/1101/B3*0101 | 0302/1101/B3*0101 | 0302/1101/B3*0101 |
| 110 | W132 | CIN1 | Negative | 0303/0601 | 1501/1301 | *0101 | 0 | *0201/2 | 0 | 0 | 0601/1501** | 0303/1301 | 0601/1501/B5*0201(2) | 0303/1301/B3*0101 |
| 111 | W134 | CIN3 | 16H | 0201/0301 | 0401/DR7 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0201/0701(2) | 0301/0401/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 112 | W135 | CIN3 | 16H | 0201/0301 | 0401/DR7 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0201/0701(2) | 0301/0401/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 113 | W137 | CIN3 | 16H | 0301/0301 | 0103/0401 | 0 | DR53 | 0 | 0301/0103 | 0301/0401 | 0301/0103** | 0301/0401 | 0301/0103/0 | 0301/0401/B4*0101(2) |
| 114 | W143 | CIN3 | 33H | 0302/0502 | 0403/1601 | 0 | DR53 | 0201/2 | 0 | 0 | 0302/0403 | 0502/1601 | 0302/0403/B4*0101(2) | 0502/1601/B5*0201(2) |
| 115 | W147 | CIN3 | 16H | 0501/0604 | 0101/1302 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0101 |
| 116 | W148 | CIN1 | Negative | 0301/0601 | 1502/1101 | *0202 | 0 | *0102 | 0 | 0 | 0601/1502 | 0301/1101 | 0601/1502/B5*0102 | 0301/1101/B3*0202 |
| 117 | W15 | CIN3 | 16H | 0302/0601 | 1501/0401 | 0 | DR53 | *0101 | 0 | 0 | 0601/1501** | 0302/0401 | 0601/1501/B5*0101 | 0302/0401/B4*0101 |
| 118 | W154 | CIN3 | 16H | 0201/0301 | 0301/1101 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1101 | 0201/0301/B3*0101 | 0301/1101/B3*0101 |
| 119 | W158 | CIN1 | Negative | 0302/0303 | 1301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0303/1301 | 0302/0401 | 0303/1301/B3*0101 | 0302/0401/B4*0101 |
| 120 | W164 | CIN1 | Negative | 0502/0604 | 1301/1301 | *0101 | 0 | 0 | 0502/1301 | 0604/1301 | 0502/1301* | 0604/1301 | 0502/1301/B3*0101 | 0604/1301/B3*0101 |
| 121 | W169 | CIN1 | 16H | 0201/0603 | 0301/1301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1301 | 0201/0301/B3*0202 | 0603/1301/B3*0202 |
| 122 | W165 | CIN3 | 16H | 0501/0606 | 0101/DR9 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0606/0901* | 0501/0101/0 | 0601/0901/B4*0101(2) |
| 123 | W166 | CIN3 | 31H | 0301/0301 | 1101/1104 | *0201/0202 | 0 | 0 | 0301/1101 | 0301/1104 | 0301/1101 | 0301/1104 | 0301/1101/B3*0201 | 0301/1104/B3*0202 |
| 124 | W18 | CIN3 | 16I | 0201/0302 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0401 | 0201/0301/B3*0101 | 0302/0401/B4*0101(2) |
| 125 | W180 | CIN3 | 16H | 0301/0601 | 1501/1304 | *0202 | 0 | *0201/2 | 0 | 0 | 0601/1501** | 0301/1304** | 0601/1501/B5*0201(2) | 0301/1304/B3*0202 |
| 126 | W182 | CIN3 | 16H | 0302/0603 | 1101/0404 | *0202 | DR53 | 0 | 0 | 0 | 0603/1101** | 0302/0404 | 0603/1101/B3*0202 | 0302/0404/B4*0101(2) |
| 127 | W186 | CIN3 | 16H | 0302/0302 | 0403/0401 | 0 | DR53 | 0201/2 | 0302/0403 | 0302/0401 | 0302/0403 | 0302/0401 | 0302/0403/B4*0101(2) | 0302/0401/B4*0101(2) |
| 128 | W191 | CIN1 | Negative | 0301/0601 | DR7/1502 | 0 | DR53 | *0102 | 0 | 0 | 0301/0701(2)* | 0601/1502 | 0301/0701(2)/B4*0101(2) | 0601/1502/B5*0102 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
|-----|-------|------|----------|------------|-------------|------------|------|-------|--------|----------------|----------------|-------------------|-------------------------|-------------------------|-------------------------|
| 129 | W198 | CIN3 | 16H | 0303/0604 | DR10/1301 | *0202 | 0 | 0 | 0 | 0 | 0.0604/1301** | 0303/1001* | 0604/1301/B3*0202 | 0303/1001/0 | |
| 130 | MP198 | CIN3 | 16H | 0301/0201 | 0301/DR7 | *0202 | DR53 | 0 | 0 | 0 | 0.0201/0301 | 0301/0701(2)* | 0201/0301/B3*0202 | 0301/0701(2)/B4*0101(2) | |
| 131 | W199 | CIN3 | 16L | 0201/0201 | 0301/0301 | *0101/0202 | 0 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0202 | |
| 132 | W2 | CIN3 | 33H | 0302/0303 | DR7/DR7 | 0 | DR53 | 0 | 0 | 0302/0701(2) | 0303/0701(2) | 0302/0701(2)** | 0303/0701(2) | 0302/0701(2)/B4*0101(2) | |
| 133 | W200 | CIN3 | 16H | 05032/0604 | 1302/1302 | *0301 | 0 | 0 | 0 | 05032/1302 | 0604/1302 | 05032/1302* | 0604/1302 | 05032/1302/B3*0301 | |
| 134 | W21 | CIN1 | Negative | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 | |
| 135 | W22 | CIN1 | Negative | 0302/0302 | 0801/0802 | 0 | 0 | 0 | 0 | 0302/0801 | 0302/0802 | 0302/0801* | 0302/0802* | 0302/0801/0 | |
| 136 | W24 | CIN1 | 16L | 0302/0302 | 0401/0404 | 0 | DR53 | 0 | 0 | 0302/0401 | 0302/0404 | 0302/0401 | 0302/0404 | 0302/0401/B4*0101 | |
| 137 | W26 | CIN1 | Negative | 05031/0604 | 1302/1401 | *0301/0101 | 0 | 0 | 0 | 05031/1401 | 0604/1302 | 05031/1401 | 0604/1302/B3*0301 | 05031/1401/B3*0101 | |
| 138 | W27 | CIN1 | Negative | 0201/0302 | 0301/0402 | *0101 | DR53 | 0 | 0 | 0 | 0.0201/0301 | 0302/0402 | 0201/0301/B3*0101 | 0302/0402/B4*0101(2) | |
| 139 | W3 | CIN1 | 16L | 0301/0303 | 1301/1101 | *0202 | 0 | 0 | 0 | 0.0301/1101 | 0303/1301 | 0301/1101/B3*0202 | 0303/1301/B3*0202 | 0303/1301/B3*0202 | |
| 140 | W30 | CIN1 | Negative | 0501/0201 | 0301/0101 | *0202 | 0 | 0 | 0 | 0.0201/0301 | 0501/0101 | 0201/0301/B3*0202 | 0501/0101/0 | 0501/0101/0 | |
| 141 | W32 | CIN3 | 16H | 0201/0603 | 0301/1104 | *0202 | 0 | 0 | 0 | 0.0201/0301 | 0603/1104** | 0201/0301/B3*0202 | 0603/1104/B3*0202 | 0603/1104/B3*0202 | |
| 142 | W33 | CIN3 | 16H | 0302/0302 | DR7/0406 | 0 | DR53 | 0 | 0 | 0 | 0.0302/0406* | 0302/0701(2)** | 0302/0701(2)/B4*0101 | 0302/0406/B4*0101 | |
| 143 | W34 | CIN3 | 16H | 0602/0201 | 1501/DR7 | 0 | DR53 | *0101 | 0 | 0 | 0.0602/1501 | 0201/0701(2) | 0602/1501/B5*0101 | 0201/0701(2)/B4*0101(2) | |
| 144 | W37 | CIN1 | Negative | 0402/0402 | 1401/0801 | *0101 | 0 | 0 | 0 | 0.0402/1401 | 0402/0801** | 0402/1401/B3*0101 | 0402/0801/0 | 0402/0801/0 | |
| 145 | W39 | CIN3 | 16L | 0301/0301 | 1101/1101 | *0101 | 0 | 0 | 0 | 0.0301/1101 | 0301/1101 | 0301/1101 | 0301/1101/B3*0101 | 0301/1101/B3*0101 | |
| 146 | W4 | CIN3 | 16L | 0201/0201 | 0301/DR9 | *0202 | DR53 | 0 | 0 | 0.0201/0301 | 0201/0901 | 0201/0301** | 0201/0301/B3*0202 | 0201/0901/B4*0101(2) | |
| 147 | W41 | CIN3 | 16L | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0 | 0.0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 | |
| 148 | W43 | CIN1 | Negative | 05032/0601 | 1501/1501 | 0 | 0 | 0 | 0201/2 | 05032/1501 | 0601/1501 | 05032/1501** | 05032/1501/B5*0202(2) | 0601/1501/B5*0201(2) | |
| 149 | W45 | CIN3 | 16H | 0504/0605 | 1401/1101 | *0201 | 0 | 0 | 0 | 0.0504/1401** | 0605/1101* | 0504/1401/B3*0201 | 0605/1101/B3*0201 | 0605/1101/B3*0201 | |
| 150 | W5 | CIN3 | 16H | 0301/0302 | 0401/1101 | *0202 | DR53 | 0 | 0 | 0 | 0.0301/1101 | 0302/0401 | 0301/1101/B3*0202 | 0302/0401/B3*0202 | |
| 151 | W51 | CIN1 | 16L | 0501/0501 | 0101/0102 | 0 | 0 | 0 | 0 | 0.0501/0101 | 0501/0102 | 0501/0101 | 0501/0102/0 | 0501/0102/0 | |
| 152 | W52 | CIN3 | 16H | 0301/0301 | 0302/0103 | *0101 | 0 | 0 | 0 | 0.0301/0103 | 0301/0302 | 0301/0103** | 0301/0302* | 0301/0103/0 | |
| 153 | W58 | CIN1 | 16L | 0603/0604 | 1101/1301 | *0101 | 0 | 0 | 0 | 0.0603/1101** | 0604/1301** | 0603/1101/B3*0101 | 0604/1301/B3*0101 | 0604/1301/B3*0101 | |
| 154 | W6 | CIN1 | Negative | 0201/0601 | 1501/0301 | *0101 | 0 | *0101 | 0 | 0 | 0.0601/1501** | 0201/0301 | 0601/1501/B5*0101 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 155 | W60 | CIN1 | 16L | 0501/0604 | 0101/1402 | *0202 | 0 | 0 | 0 | 0 | 0.0501/0101 | 0604/1402* | 0501/0101/0 | 0604/1402/B3*0202 | |
| 156 | W64 | CIN3 | 16L | 0501/0501 | 0101/0103 | 0 | 0 | 0 | 0 | 0.0501/0101 | 0501/0103 | 0501/0101 | 0501/0103 | 0501/0103/0 | |
| 157 | W66 | CIN3 | Negative | 0302/0605 | 0403/1302 | *0101 | DR53 | 0 | 0 | 0 | 0.0302/0403 | 0605/1302 | 0302/0403/B4*0101 | 0605/1302/B3*0101 | 0605/1302/B3*0101 |
| 158 | W7 | CIN3 | 16L | 0301/0501 | 0101/1101 | *0101 | 0 | 0 | 0 | 0 | 0.0501/0101 | 0301/1101 | 0501/0101/0 | 0301/1101/B3*0101 | 0301/1101/B3*0101 |
| 159 | W70 | CIN1 | Negative | 0302/0604 | 1302/0403 | *0202 | DR53 | 0 | 0 | 0 | 0.0604/1302 | 0302/0403 | 0604/1302/B3*0202 | 0302/0403/B4*0101(2) | 0302/0403/B4*0101(2) |
| 160 | W72 | CIN3 | 33L | 0201/0502 | DR10/0301 | *0202 | 0 | 0 | 0 | 0 | 0.0502/1001* | 0201/0301 | 0502/1001/0 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 161 | W74 | CIN1 | Negative | 0301/0301 | 08042/08042 | *0202 | 0 | 0 | 0 | 0.0301/08042 | 0301/08042** | 0301/08042** | 0301/08042/B3*0202* | 0301/08042/0 | 0301/08042/0 |
| 162 | W78 | CIN3 | Negative | 0301/0302 | 0802/0401 | 0 | DR53 | 0 | 0 | 0 | 0.0301/08042** | 0302/0401 | 0301/0802/0 | 0302/0401/B4*0101(2) | 0302/0401/B4*0101(2) |
| 163 | W8 | CIN3 | Negative | 0201/0201 | 0301/1406 | *0202 | 0 | 0 | 0 | 0.0201/0301 | 0201/1406 | 0201/0301 | 0201/1406* | 0201/0301/B3*0202 | 0201/1406/B3*0202 |
| 164 | W80 | CIN3 | 16H | 0201/0301 | 0801/DR9 | *0201 | 0 | 0 | 0 | 0 | 0.0201/0801* | 0301/0901 | 0201/0801/B3*0201* | 0301/0901/0* | 0301/0901/0* |
| 165 | W81 | CIN1 | 33H | 0302/0302 | 0401/0403 | 0 | DR53 | 0 | 0 | 0.0302/0401 | 0302/0403 | 0302/0401 | 0302/0403 | 0302/0401/B4*0101(2) | 0302/0403/B4*0101(2) |
| 166 | W82 | CIN3 | 16H | 0201/0604 | 0301/1302 | *0301/0202 | 0 | 0 | 0 | 0 | 0.0201/0301 | 0604/1302 | 0201/0301/B3*0202 | 0604/1302/B3*0301 | 0604/1302/B3*0301 |
| 167 | W84 | CIN1 | Negative | 0402/0402 | 0301/0803 | *0201 | 0 | 0 | 0 | 0.0402/0301 | 0402/0803 | 0402/0301* | 0402/0803 | 0402/0301/B3*0201 | 0402/0803/0 |
| 168 | W86 | CIN3 | 16H | 0502/0503 | 1601/1602 | 0 | 0 | 0 | 0201/2 | 0 | 0 | 0.0502/1601 | 05031/1602* | 0502/1601/B5*0201(2) | 05031/1602/B5*0201(2) |
| 169 | W87 | CIN1 | 16H | 0201/0601 | DR7/DR9 | 0 | DR53 | 0 | 0 | 0 | 0.0201/0701(2) | 0601/0901* | 0201/0701(2)/B4*0101(2) | 0601/0901/B4*0101(2) | 0601/0901/B4*0101(2) |
| 170 | W88 | CIN1 | 16L | 0201/0302 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0.0201/0301 | 0302/0401 | 0201/0301/B3*0101 | 0302/0401/B4*0101(2) | 0302/0401/B4*0101(2) |
| 171 | W9 | CIN3 | 16H | 0302/0302 | DR7/DR7 | 0 | DR53 | 0 | 0 | 0.0302/0701(2) | 0302/0701(2) | 0302/0701(2)** | 0302/0701(2)/B4*0101(2) | 0302/0701(2)/B4*0101(2) | 0302/0701(2)/B4*0101(2) |
| 172 | W90 | CIN3 | 16L | 0301/0604 | 0401/DR9 | 0 | DR53 | 0 | 0 | 0 | 0.0301/0401 | 0604/0901* | 0301/0401/B4*0101(2) | 0604/0901/B4*0101(2) | 0604/0901/B4*0101(2) |
| 173 | W91 | CIN3 | 31L | 0302/0603 | 0401/1101 | *0202 | DR53 | 0 | 0 | 0 | 0.0302/0401 | 0603/1101** | 0302/0401/B4*0101(2) | 0603/1101/B3*0202 | 0603/1101/B3*0202 |
| 174 | W92 | CIN1 | 16L | 0501/0502 | 0101/1402 | *0101 | 0 | 0 | 0 | 0 | 0.0501/0101 | 0502/1402 | 0501/0101/0 | 0502/1402/B3*0101 | 0502/1402/B3*0101 |
| 175 | W96 | CIN3 | 16L 18L | 0201/0601 | 0301/1502 | *0202 | 0 | *0102 | 0 | 0 | 0.0201/0301 | 0601/1502 | 0201/0301/B3*0202 | 0601/1502/B5*0102 | 0601/1502/B5*0102 |
| 176 | W97 | CIN1 | Negative | 0302/0301 | 0103/0401 | 0 | DR53 | 0 | 0 | 0 | 0.0301/0103** | 0302/0401 | 0301/0103/0 | 0302/0401/B4*0101(2) | 0302/0401/B4*0101(2) |
| 177 | W98 | CIN3 | 16H | 05031/0502 | 1401/1401 | *0202 | 0 | 0 | 0 | 0 | 0.05031/1401 | 0502/1401* | 05031/1401/B3*0202 | 0502/1401/B3*0202 | 0502/1401/B3*0202 |
| 178 | | | | | | | | | | | | | | | |
| 179 | | | | | | | | | | | | | | | |
| 180 | | | | | | | | | | | | | | | |
| 181 | | | | | | | | | | | | | | | |
| 182 | | | | | | | | | | | | | | | |
| 183 | | | | | | | | | | | | | | | |
| 184 | | | | | | | | | | | | | | | |
| 185 | | | | | | | | | | | | | | | |
| 186 | | | | | | | | | | | | | | | |
| 187 | | | | | | | | | | | | | | | |
| 188 | | | | | | | | | | | | | | | |
| 189 | | | | | | | | | | | | | | | |
| 190 | | | | | | | | | | | | | | | |
| 191 | | | | | | | | | | | | | | | |
| 192 | | | | | | | | | | | | | | | |

**APPENDIX II: FULL HLA DR-DQ RESULTS ON CONTROL
POPULATION**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----|-----------|------------|-----------|------------|------------|---------|------------------|------------------|---------------|---------------|-------------------------|-------------------------|
| | Sample No | DQB1 | DRB1 | DRB3(DRS2) | DRB4(DRS3) | DRB5 | Inferred Haplo.1 | Inferred Haplo.2 | CephDQ-DRB1.1 | CephDQ-DRB1.2 | DQ-DRB1-DRB345.1 | DQ-DRB1-DRB345.2 |
| 1 | MP508 | 0301/0301 | 1104/1305 | *0202 | 0 | 0 | 0301/1104 | 0301/1305 | 0301/1104 | 0301/1305 | 0301/1104/B3*0202 | 0301/1305/B3*0202 |
| 2 | MP1 | 0201/0301 | 0301/1101 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1101 | 0201/0301/B3*0202 | 0301/1101/B3*0202 |
| 3 | MP10 | 0301/0501 | 1101/0101 | *0202 | 0 | 0 | 0 | 0 | 0301/1101 | 0501/0101 | 0301/1101/B3*0202 | 0501/0101/0 |
| 4 | MP100 | 0301/0601 | 1501/0401 | 0 | DR53 | *0201/2 | 0 | 0 | 0601/1501** | 0301/0401 | 0601/1501/B5*0201(2) | 0301/0401/B4*0101(2) |
| 5 | MP1002 | 0401/0501 | 0101/0404 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0401/0404* | 0501/0101/0 | 0401/0404/B4*0101(2) |
| 6 | MP1003 | 0201/0602 | DR9/1501 | 0 | DR53 | *0101 | 0 | 0 | 0602/1501 | 0201/0901* | 0602/1501/B5*0101 | 0201/0901/B4*0101(2) |
| 7 | MP1004 | 0501/0604 | 0101/1301 | *0202 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0202 |
| 8 | MP1005 | 0501/0502 | 0101/DR7 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0502/0701(2)* | 0501/0101/0 | 0502/0701(2)/B4*0101(2) |
| 9 | MP1007 | 0201/0602 | 0301/1406 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0602/1406* | 0201/0301/B3*0202 | 0602/1406/B3*0202 |
| 10 | MP1008 | 0501/0604 | 0102/1302 | *0301 | 0 | 0 | 0 | 0 | 0501/0102 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0301 |
| 11 | MP1014 | 0201/05031 | 0301/1401 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 05031/1401 | 0201/0301/B3*0101 | 05031/1401/B3*0101 |
| 12 | MP1015 | 0201/0604 | 0301/1301 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1301** | 0201/0301/B3*0101 | 0604/1301/B3*0101 |
| 13 | MP102 | 0201/0501 | 0410/0101 | 0 | DR53 | 0 | 0 | 0 | 0201/0410* | 0501/0101 | 0201/0401/B4*0101(2) | 0501/0101/0 |
| 14 | MP103 | 05031/0402 | 0801/DR9 | 0 | DR53 | 0 | 0 | 0 | 0402/0801** | 05031/0901* | 0402/0801/0 | 05031/0901/B4*0101(2) |
| 15 | MP105 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 16 | MP106 | 0301/0601 | 0401/1501 | 0 | DR53 | *0101 | 0 | 0 | 0301/0401 | 0601/1501 | 0301/0401/B4*0101(2) | 0601/1501/B5*0101 |
| 17 | MP107 | 0401/05032 | 1401/DR9 | *0202 | DR53 | 0 | 0 | 0 | 0401/0901* | 0503/1401 | 0401/0901/B4*0101(2) | 0503/1401/B3*0202 |
| 18 | MP11 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 19 | MP111 | 0201/0605 | 1501/0301 | 0 | 0 | 0201/2 | 0 | 0 | 0201/0301 | 0605/1501* | 0201/0301/0* | 0605/1501/B5*0201(2) |
| 20 | MP112 | 0201/0601 | 1502/0301 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1502 | 0201/0301/B3*0101 | 0601/1502/B5*0101 |
| 21 | MP113 | 0501/0604 | 1301/0101 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 22 | MP114 | 0302/0602 | 1501/0404 | 0 | DR53 | *0101 | 0 | 0 | 0302/0404 | 0602/1501 | 0302/0404/B4*0101 | 0602/1501/B5*0101 |
| 23 | MP115 | 0302/0201 | 0404/DR7 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0404 | 0201/0701(2)/B4*0101(2) | 0302/0404/B4*0101 |
| 24 | MP117 | 0201/0601 | 0301/DR9 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0601/0901* | 0201/0301/B3*0202 | 0601/0901/B4*0101 |
| 25 | MP1185 | 0302/0402 | 0407/DR10 | 0 | DR53 | 0 | 0 | 0 | 0302/0407 | 0402/1001* | 0302/0407/B4*0101 | 0402/1001/0 |
| 26 | MP119 | 0501/0603 | DR10/1104 | *0202 | 0 | 0 | 0 | 0 | 0501/1001 | 0603/1104** | 0501/1001/0 | 0603/1104/B3*0202 |
| 27 | MP1193 | 0302/0302 | 0401/0404 | 0 | DR53 | 0 | 0302/0401 | 0302/0404 | 0302/0401 | 0302/0404 | 0302/0401/B4*0101(2) | 0302/0404/B4*0101(2) |
| 28 | MP120 | 05032/0601 | DR10/1502 | 0 | 0 | *0102 | 0 | 0 | 0601/1502 | 05032/1001* | 0602/1502/B5*0102 | 05032/1001/0 |
| 29 | MP122 | 0502/05031 | 1401/DR7 | 0 | DR53 | 0 | 0 | 0 | 05031/1401 | 0502/0701(2)* | 05031/1401/0* | 0502/0701(2)/B4*0101(2) |
| 30 | MP123 | 0501/0501 | 0101/0103 | 0 | 0 | 0 | 0501/0101 | 0501/0103 | 0501/0101 | 0501/0103 | 0501/0101/0 | 0501/0103/0 |
| 31 | MP124 | 0301/0301 | 1104/DR9 | *0101 | DR53 | 0 | 0301/1104 | 0301/0901 | 0301/1104 | 0301/0901* | 0301/1104/B3*0101 | 0301/0901/B4*0101(2) |
| 32 | MP125 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301 | 0501/0101/0 | 0604/1301/B3*0101 |
| 33 | MP1257 | 0402/0501 | 0101/0803 | 0 | 0 | 0 | 0 | 0 | 0501/0101 | 0402/0803** | 0501/0101/0 | 0402/0803/0 |
| 34 | MP1259 | 0301/0302 | DR9/0401 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0302/0901* | 0301/0401/B4*0101(2) | 0302/0901/B4*0101(2) |
| 35 | MP126 | 0301/0201 | 1501/0103 | 0 | 0 | *0101 | 0 | 0 | 0301/0103** | 0201/1501* | 0301/0103/0 | 0201/1501/B5*0101 |
| 36 | MP1260 | 0303/0201 | 1301/0301 | *0101 | 0 | 0 | 0 | 0 | 0303/1301 | 0201/0301 | 0303/1301/B3*0101 | 0201/0301/B3*0101 |
| 37 | MP1262 | 0302/0302 | 0401/0403 | 0 | DR53 | 0 | 0302/0401 | 0302/0403 | 0302/0401 | 0302/0403 | 0302/0401/B4*0101(2) | 0302/0403/B4*0101(2) |
| 38 | MP127 | 0601/0601 | 1501/1502 | 0 | 0 | *0101/2 | 0601/1501 | 0601/1502 | 0601/1501** | 0601/1502 | 0601/1501/B5*0101 | 0601/1502/B5*0102 |
| 39 | MP1401 | 0201/0601 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | MP1420 | 0301/0301 | 0401/0103 | 0 | DR53 | 0 | 0301/0401 | 0301/0103 | 0301/0401 | 0301/0103** | 0301/0401/B4*0101(2) | 0301/0103/0 |
| 41 | MP1421 | 0201/0402 | 0301/0803 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0402/0803** | 0201/0301/B3*0101 | 0402/0803/0 |
| 42 | MP1422 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 43 | MP1424 | 0402/0604 | 1302/0801 | *0301 | 0 | 0 | 0 | 0 | 0402/0801** | 0604/1302 | 0402/0801/0 | 0604/1302/B3*0301 |
| 44 | MP146 | 0201/0302 | 0301/0403 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0403 | 0201/0301/B3*0101 | 0302/0403/B4*0101 |
| 45 | MP1461 | 0201/0603 | 1301/DR7 | *0101 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0603/1301 | 0201/0701(2)/B4*0101(2) | 0603/1301/B3*0101 |
| 46 | MP1462 | 0303/0501 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0303/1301 | 0501/0101/0 | 0303/1301/B3*0101 |
| 47 | MP1465 | 0302/0603 | 1301/0401 | *0202 | DR53 | 0 | 0 | 0 | 0302/0401 | 0603/1301 | 0302/0401/B4*0101(2) | 0603/1301/B3*0202 |
| 48 | MP1466 | 0301/05031 | 1401/1201 | *0202 | 0 | 0 | 0 | 0 | 0301/1201 | 05031/1401 | 0301/1201/B3*0202 | 05031/1401/B3*0202 |
| 49 | MP1467 | 0201/0303 | DR7/DR7 | 0 | DR53 | 0 | 0201/0701(2) | 0303/0701(2) | 0201/0701(2) | 0303/0701(2) | 0201/0701(2)/B4*0101(2) | 0303/0701(2)/B4*0101(2) |
| 50 | MP1468 | 0302/0301 | 0404/0404 | 0 | DR53 | 0 | 0302/0404 | 0301/0404 | 0302/0404 | 0301/0404* | 0302/0404/B4*0101(2) | 0301/0404/B4*0101(2) |
| 51 | MP1469 | 0501/0501 | 0101/DR7 | 0 | DR53 | 0 | 0501/0101 | 0501/0701(2) | 0501/0101 | 0501/0701(2)* | 0501/0101/0 | 0501/0701(2)/B4*0101(2) |
| 52 | MP147 | 0301/0501 | 0101/0103 | 0 | 0 | 0 | 0 | 0 | 0301/0103** | 0501/0101 | 0301/0103/0 | 0501/0101/0 |
| 53 | MP1470 | 0201/0201 | 0301/DR7 | *0101 | DR53 | 0 | 0201/0301 | 0201/0701(2) | 0201/0301 | 0201/0701(2) | 0201/0301/B3*0101 | 0201/0701(2)/B4*0101(2) |
| 54 | MP1471 | 0301/0301 | 0103/0401 | 0 | DR53 | 0 | 0301/0103 | 0301/0401 | 0301/0103** | 0301/0401 | 0301/0103/0 | 0301/0401/B4*0101(2) |
| 55 | MP1472 | 0201/0603 | 0301/1301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1301 | 0201/0301/B3*0202 | 0603/1301/B3*0202 |
| 56 | MP1473 | 0402/0604 | 1301/0801 | *0202 | 0 | 0 | 0 | 0 | 0402/0801** | 0604/1301** | 0402/0801/0 | 0604/1301/B3*0202 |
| 57 | MP1474 | 0501/0605 | 0101/1302 | *0201 | 0 | 0 | 0 | 0 | 0501/0101 | 0605/1302* | 0501/0101/0 | 0605/1302/B3*0201 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|--------|------------|-----------|------------|------|--------|--------------|--------------|--------------|---------------|-------------------------|-------------------------|
| 59 | MP1475 | 0402/0501 | 0801/DR7 | 0 | DR53 | 0 | 0 | 0 | 0402/0801** | 0501/0701(2)* | 0402/0801/0 | 0501/0701(2)/B4*0101(2) |
| 60 | MP1476 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 61 | MP148 | 0302/0302 | 1101/DR7 | *0202 | DR53 | 0 | 0302/1101 | 0302/0701(2) | 0302/1101 | 0302/0701(2) | 0302/1101/B3*0202 | 0302/0701(2)/B4*0101(2) |
| 62 | MP149 | 0201/0301 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0301/0401 | 0201/0301/B3*0101 | 0301/0401/B4*0101 |
| 63 | MP1496 | 0402/05031 | 0803/1401 | *0202 | 0 | 0 | 0 | 0 | 0402/0803** | 05031/1401 | 0402/0803/0 | 05031/1401/B3*0202 |
| 64 | MP1497 | 0402/0604 | 1301/0801 | *0101 | 0 | 0 | 0 | 0 | 0402/0801 | 0604/1301 | 0402/0801/0 | 0604/1301/B3*0101 |
| 65 | MP1498 | 0201/0601 | 1501/0301 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501 | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 66 | MP1499 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 67 | MP15 | 0301/0501 | 0101/0401 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0501/0101 | 0301/0401/B4*0101(2) | 0501/0101 |
| 68 | MP150 | 0201/0603 | 1103/0301 | *0101/0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0601/1103* | 0201/0301/B3*0101 | 0601/1103/B3*0202 |
| 69 | MP1500 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 70 | MP1503 | 0401/0501 | 0101/0401 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0401/0401** | 0501/0101/0 | 0401/0401/B4*0101(2) |
| 71 | MP1504 | 0201/0601 | 0301/1501 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 72 | MP1506 | 0301/0302 | 0404/0404 | 0 | DR53 | 0 | 0301/0404 | 0302/0404 | 0301/0404** | 0302/0404 | 0301/0404/B4*0101 | 0302/0404/B4*0101 |
| 73 | MP1507 | 0501/0604 | 0101/1302 | *0301 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0301 |
| 74 | MP1509 | 0301/0303 | 1201/1201 | *0201 | 0 | 0 | 0301/1201 | 0303/1201 | 0301/1201 | 0303/1201* | 0301/1201/B3*0201 | 0303/1201/B3*0201 |
| 75 | MP151 | 0501/0601 | 0101/1502 | 0 | 0 | *0102 | 0 | 0 | 0501/0101 | 0601/1502 | 0501/0101/0 | 0601/1502/B5*0102 |
| 76 | MP152 | 0302/0201 | 0401/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0401 | 0201/0301/B3*0101 | 0302/0401/B4*0101 |
| 77 | MP153 | 0501/0604 | 0101/1302 | *0301 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0301 |
| 78 | MP154 | 0501/0604 | 0101/0302 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/0302* | 0501/0101/0 | 0604/0302/B3*0101 |
| 79 | MP155 | 0201/0601 | 0301/1301 | *0301 | 0 | 0 | 0 | 0 | 0201/0301 | 0601/1301* | 0201/0301/B3*0301 | 0601/1301/B3*0301 |
| 80 | MP156 | 0303/0502 | 1501/DR7 | 0 | DR53 | *0101 | 0 | 0 | 0502/1501 | 0303/0701(2) | 0502/1501/B5*0101 | 0303/0701(2)/B4*0101 |
| 81 | MP157 | 0201/0301 | 0301/1202 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1202** | 0201/0301/B3*0101 | 0301/1202/B3*0101 |
| 82 | MP158 | 0302/0504 | 0404/1404 | *0101 | DR53 | 0 | 0 | 0 | 0302/0404 | 0504/1404** | 0302/0404/B4*0101 | 0504/1404/B3*0101 |
| 83 | MP159 | 0501/0604 | 0101/1302 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0101 |
| 84 | MP1591 | 0302/0201 | DR9/DR10 | 0 | DR53 | 0 | 0 | 0 | 0302/0901* | 0201/1001* | 0302/0901/B4*0101 | 0201/1001/0 |
| 85 | MP1592 | 0201/0302 | 0404/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0404 | 0201/0301/B3*0101 | 0302/0404/B4*0101 |
| 86 | MP1593 | 0302/0302 | 0404/0802 | 0 | DR53 | 0 | 0302/0404 | 0302/0802 | 0302/0404 | 0302/0802 | 0302/0404/B4*0101 | 0302/0802/0 |
| 87 | MP1596 | 0301/0301 | 0401/0404 | 0 | DR53 | 0 | 0301/0401 | 0301/0404 | 0301/0401 | 0301/0404** | 0301/0401/B4*0101 | 0301/0404/B4*0101 |
| 88 | MP1594 | 0201/0301 | 0301/1201 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1201 | 0201/0301/B3*0101 | 0301/1201/B3*0101 |
| 89 | MP1598 | 0201/0601 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 90 | MP1599 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 91 | MP16 | 0301/0402 | 1201/0801 | *0101 | 0 | 0 | 0 | 0 | 0301/1201 | 0402/0801** | 0301/1201/B3*0101 | 0402/0801/0 |
| 92 | MP160 | 0601/0603 | DR7/DR10 | 0 | DR53 | 0 | 0 | 0 | 0601/0701(2) | 0603/1001* | 0601/0701(2)/B4*0101 | 0603/1001/0 |
| 93 | MP1600 | 0501/0604 | 0101/0404 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0604/0404* | 0501/0101/0 | 0604/0404/B4*0101(2) |
| 94 | MP1601 | 0604/0604 | 1301/DR7 | *0301 | DR53 | 0 | 0604/1301 | 0604/0701(2) | 0604/1301** | 0604/0701(2)* | 0604/1301/B3*0301 | 0604/0701(2)/B4*0101(2) |
| 95 | MP1602 | 0201/0601 | 0301/DR7 | *0101 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0601/0301* | 0201/0701(2)/B4*0101(2) | 0601/0301/B3*0101 |
| 96 | MP1604 | 0401/0402 | 0401/0801 | 0 | DR53 | 0 | 0 | 0 | 0402/0801** | 0401/0401** | 0402/0801/0 | 0401/0401/B4*0101(2) |
| 97 | MP1606 | 0201/0201 | 0301/DR7 | *0101 | DR53 | 0 | 0201/0301 | 0201/0701(2) | 0201/0301 | 0201/0701(2) | 0201/0301/B3*0101 | 0201/0701(2)/B4*0101(2) |
| 98 | MP1607 | 0501/0604 | 0101/1302 | *0201 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0201 |
| 99 | MP1608 | 0603/0605 | 1301/DR7 | *0202 | 0 | 0 | 0 | 0 | 0603/1301 | 0605/0701(2)* | 0603/1301/B3*0202 | 0605/0701(2)/B4*0101(2) |
| 100 | MP161 | 0601/0601 | 1501/1301 | 0 | 0 | *0101 | 0601/1501 | 0601/1501 | 0601/1501** | 0601/1501** | 0601/1501/B5*0101 | 0601/1501/B5*0101 |
| 101 | MP163 | 0501/0601 | 1501/0101 | 0 | 0 | 0201/2 | 0 | 0 | 0501/0101 | 0601/1501** | 0501/0101/0 | 0601/1501/B5*0201(2) |
| 102 | MP1633 | 0201/0201 | 0301/DR10 | *0101 | 0 | 0 | 0201/0301 | 0201/1001 | 0201/0301 | 0201/1001* | 0201/0301/B3*0101 | 0201/1001/0 |
| 103 | MP1634 | 0502/05031 | 1601/1401 | *0101 | 0 | 0201/2 | 0 | 0 | 0502/1601 | 05031/1401 | 0502/1601/B5*0201(2) | 05031/1401/B3*0101 |
| 104 | MP1635 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 105 | MP1636 | 0302/0601 | 0404/1501 | 0 | DR53 | *0101 | 0 | 0 | 0302/0404 | 0601/1501** | 0302/0404/B4*0101 | 0601/1501/B5*0101 |
| 106 | MP1637 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 107 | MP164 | 0201/0303 | DR7/DR7 | 0 | DR53 | 0 | 0201/0701(2) | 0201/0701(2) | 0201/0701(2) | 0201/0701(2) | 0201/0701(2)/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 108 | MP165 | 0601/0603 | 1501/1301 | *0202 | 0 | *0101 | 0 | 0 | 0601/1501** | 0603/1301 | 0601/1501/B5*0101 | 0603/1301/B3*0202 |
| 109 | MP166 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 110 | MP167 | 0601/0601 | 1501/1501 | 0 | 0 | *0101 | 0601/1501 | 0601/1501 | 0601/1501** | 0601/1501** | 0601/1501/B5*0101 | 0601/1501/B5*0101 |
| 111 | MP168 | 0502/05032 | 1501/1401 | *0202 | 0 | *0101 | 0 | 0 | 0502/1501 | 05032/1401 | 0502/1501/B5*0101 | 05032/1401/B3*0202 |
| 112 | MP169 | 0501/0504 | 0101/1401 | *0202 | 0 | 0 | 0 | 0 | 0501/0101 | 0504/1401** | 0501/0101/0 | 0504/1401/B3*0202 |
| 113 | MP17 | 0201/0303 | 0301/DR7 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0303/0701(2) | 0201/0301/B3*0101 | 0303/0701(2)/B4*0101(2) |
| 114 | MP170 | 0201/0603 | 0301/1301 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1301 | 0201/0301/B3*0101 | 0603/1301/B3*0101 |
| 115 | MP171 | 0301/0601 | 1104/1305 | *0202 | 0 | 0 | 0 | 0 | 0301/1104 | 0601/1305* | 0301/1104/B3*0202 | 0601/1305/B3*0202 |
| 116 | MP172 | 0201/0601 | 0301/1501 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|-------|------------|-----------|------------|------|------------|-----------|-----------|----------------|----------------|-------------------------|-------------------------|
| 117 | MP173 | 0201/0504 | DR9/DR7 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0504/0901* | 0201/0701(2)/B4*0101 | 0504/0901/B4*0101(2) |
| 118 | MP174 | 0301/0504 | 1401/0401 | *0101 | DR53 | 0 | 0 | 0 | 0301/0401 | 0504/1401** | 0301/0401/B4*0101(2) | 0504/1401/B3*0101 |
| 119 | MP175 | 0201/0601 | 1501/0302 | *0101 | 0 | 0201/2 | 0 | 0 | 0201/0302* | 0601/1501** | 0201/0302/B3*0101 | 0601/1501/B5*0201(2) |
| 120 | MP176 | 0501/0604 | 0101/0801 | 0 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/0801 | 0501/0101/0 | 0604/0801/0 |
| 121 | MP177 | 0301/0402 | DR7/DR10 | 0 | DR53 | 0 | 0 | 0 | 0301/0701(2)** | 0402/1001* | 0301/0701(2)/B4*0101(2) | 0402/1001/0 |
| 122 | MP178 | 0201/0601 | 0301/1501 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 123 | MP179 | 0504/0601 | 1501/DR10 | 0 | 0 | 0201/2 | 0 | 0 | 0601/1501 | 0504/1001* | 0601/1501/B5*0201(2) | 0504/1001/0 |
| 124 | MP18 | 0302/0201 | 0401/DR7 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0401 | 0201/0701(2)/B4*0101(2) | 0302/0401/B4*0101(2) |
| 125 | MP180 | 0201/0604 | 0301/1302 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1302 | 0201/0301/B3*0101 | 0604/1302/B3*0101 |
| 126 | MP181 | 0302/0501 | 0101/DR7 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0302/0701(2)** | 0501/0101/0 | 0302/0701(2)/B4*0101(2) |
| 127 | MP182 | 0201/0601 | 1502/0301 | *0202 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1502 | 0201/0301/B3*0202 | 0601/1502/B5*0101 |
| 128 | MP183 | 0302/0601 | 0407/1501 | 0 | DR53 | *0101 | 0 | 0 | 0302/0407** | 0601/1501** | 0302/0407/B4*0101(2) | 0601/1501/B5*0101 |
| 129 | MP184 | 0301/0201 | 0301/1101 | *0202 | 0 | 0 | 0 | 0 | 0301/1101 | 0201/0301 | 0301/1101/B3*0202 | 0201/0301/B3*0202 |
| 130 | MP185 | 0201/0601 | 0302/1502 | *0101 | 0 | *0102 | 0 | 0 | 0201/0302* | 0601/1502 | 0201/0302/B3*0101 | 0601/1502/B5*0102 |
| 131 | MP186 | 0302/0302 | 0401/0402 | 0 | DR53 | 0 | 0302/0401 | 0302/0402 | 0302/0401 | 0302/0402 | 0302/0401/B4*0101(2) | 0302/0402/B4*0101(2) |
| 132 | MP187 | 0201/0601 | 0301/1501 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 133 | MP188 | 0402/0803 | 1501/1501 | 0 | 0 | 0201/2 | 0402/1501 | 0603/1501 | 0402/1501* | 0603/1501* | 0402/1501/B5*0202(2) | 0603/1501/B5*0201(2) |
| 134 | MP189 | 05031/0504 | 0404/DR10 | 0 | DR53 | 0 | 0 | 0 | 0504/1001* | 05031/0404* | 0504/1001/0 | 05031/0404/B4*0101(2) |
| 135 | MP19 | 0302/0201 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0401 | 0201/0301/B3*0101 | 0302/0401/B4*0101(2) |
| 136 | MP190 | 0601/0603 | 1501/1301 | *0101 | 0 | *0101 | 0 | 0 | 0601/1501** | 0603/1301 | 0601/1501/B5*0101 | 0603/1301/B3*0101 |
| 137 | MP2 | 0301/0603 | 0103/1101 | *0202 | 0 | 0 | 0 | 0 | 0301/0103 | 0603/1101 | 0301/0103/0 | 0603/1101/B3*0202 |
| 138 | MP21 | 05032/0602 | 1401/1501 | *0101 | 0 | *0101 | 0 | 0 | 0602/1501 | 05032/1401 | 0602/1501/B5*0101 | 05032/1401/B3*0101 |
| 139 | MP202 | 0601/0604 | 1501/1301 | *0101 | 0 | *0101 | 0 | 0 | 0601/1501** | 0604/1301** | 0601/1501/B5*0101 | 0604/1301/B3*0101 |
| 140 | MP203 | 0301/0302 | 1101/0401 | *0201 | DR53 | 0 | 0 | 0 | 0302/0401 | 0301/1101 | 0302/0401/B4*0101(2) | 0301/1101/B3*0201 |
| 141 | MP207 | 0303/0601 | DR9/1501 | 0 | DR53 | *0101 | 0 | 0 | 0303/0901** | 0601/1501** | 0303/0901/B4*0101(2) | 0601/1501/B5*0101 |
| 142 | MP208 | 0401/0604 | 0101/DR7 | 0 | DR53 | 0 | 0 | 0 | 0401/0701(2)* | 0604/0101* | 0401/0701/B4*0101(2) | 0604/0101/0 |
| 143 | MP209 | 0301/0504 | 1401/0103 | *0202 | 0 | 0 | 0 | 0 | 0301/0103** | 0504/1401** | 0301/0103/0 | 0504/1401/B3*0202 |
| 144 | MP210 | 0302/0601 | 1501/0404 | 0 | DR53 | *0101 | 0 | 0 | 0302/0404 | 0601/1501** | 0302/0404/B4*0101(2) | 0302/0404/B4*0101(2) |
| 145 | MP211 | 0601/0601 | 1501/1502 | 0 | 0 | *0101/0102 | 0601/1501 | 0601/1502 | 0601/1501** | 0601/1502 | 0601/1501/B5*0101 | 0601/1502/B5*0102 |
| 146 | MP212 | 0603/0604 | 0302/1407 | *0101 | 0 | 0 | 0 | 0 | 0603/0302* | 0604/1407* | 0603/0302/B3*0101 | 0604/1407/B3*0101 |
| 147 | MP22 | 0501/0501 | 0101/0101 | 0 | 0 | 0 | 0501/0101 | 0501/0101 | 0501/0101 | 0501/0101 | 0501/0101/0 | 0501/0101/0 |
| 148 | MP23 | 0201/0302 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0401 | 0201/0301/B3*0101 | 0302/0401/B4*0101(2) |
| 149 | MP234 | 0301/0402 | 1304/1304 | *0202 | 0 | 0 | 0301/1304 | 0402/1304 | 0301/1304** | 0402/1304** | 0301/1304/B3*0202 | 0402/1304/B3*0202 |
| 150 | MP236 | 0201/0302 | 0301/0402 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0402 | 0201/0301/B3*0202 | 0302/0402/B4*0101(2) |
| 151 | MP237 | 0603/0604 | 1301/1302 | *0202/0301 | 0 | 0 | 0 | 0 | 0603/1301 | 0604/1302 | 0603/1301/B3*0202 | 0604/1302/B3*0301 |
| 152 | MP238 | 0402/0501 | 0801/0802 | *0101 | 0 | 0 | 0 | 0 | 0402/0801 | 0501/0801* | 0402/0801/B3*0101 | 0501/0801/0 |
| 153 | MP239 | 0201/0601 | 0302/0302 | *0101 | 0 | 0 | 0201/0302 | 0601/0302 | 0201/0302* | 0601/0302* | 0201/0302/B3*0101 | 0601/0302/B3*0101 |
| 154 | MP24 | 0201/0602 | 0301/1406 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0602/1406* | 0201/0301/B3*0202 | 0602/1406/B3*0202 |
| 155 | MP240 | 0601/0601 | 1501/1502 | 0 | 0 | 0201/2 | 0601/1501 | 0601/1502 | 0601/1501 | 0601/1502 | 0601/1501/B5*0201(2) | 0601/1502/B5*0201(2) |
| 156 | MP241 | 0302/0603 | 0404/1301 | *0101 | DR53 | 0 | 0 | 0 | 0302/0404 | 0603/1301 | 0302/0404/B4*0101(2) | 0603/1301/B3*0101 |
| 157 | MP242 | 0201/0603 | 0301/DR10 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1001* | 0201/0301/B3*0101 | 0603/1001/0 |
| 158 | MP243 | 0302/0504 | 0401/1401 | *0101 | DR53 | 0 | 0 | 0 | 0302/0401 | 0504/1401** | 0302/0401/B4*0101(2) | 0504/1401/B3*0101 |
| 159 | MP244 | 0201/0801 | 1501/DR9 | 0 | DR53 | *0101 | 0 | 0 | 0601/1501 | 0201/0901* | 0601/1501/B5*0101 | 0201/0901/B4*0101(2) |
| 160 | MP246 | 0302/0201 | 0301/0404 | *0101 | DR53 | 0 | 0 | 0 | 0302/0404 | 0201/0301 | 0302/0404/B4*0101(2) | 0201/0301/B3*0101 |
| 161 | MP249 | 0201/0302 | DR7/1101 | *0202 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/1101 | 0201/0701(2)/B4*0101(2) | 0302/1101/B3*0202 |
| 162 | MP250 | 0502/0605 | 1404/0802 | *0202 | 0 | 0 | 0 | 0 | 0502/1404* | 0605/0802* | 0502/1404/B3*0202 | 0605/0802/0 |
| 163 | MP251 | 0301/0302 | 1104/0403 | *0101 | DR53 | 0 | 0 | 0 | 0301/1104 | 0302/0403 | 0301/1104/B3*0101 | 0302/0403/B4*0101 |
| 164 | MP252 | 0501/0603 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0603/1301 | 0501/0101/0 | 0603/1301/B3*0101 |
| 165 | MP254 | 0302/0401 | 0404/0404 | 0 | DR53 | 0 | 0302/0404 | 0401/0404 | 0302/0404 | 0401/0404* | 0302/0404/B4*0101(2) | 0401/0404/B4*0101(2) |
| 166 | MP256 | 0201/0301 | 0301/0401 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0301/0401 | 0201/0301/B3*0202 | 0301/0401/B4*0101(2) |
| 167 | MP257 | 0302/0504 | 0402/1401 | *0101 | DR53 | 0 | 0 | 0 | 0302/0402 | 0504/1401** | 0302/0402/B4*0101(2) | 0504/1401/B3*0101 |
| 168 | MP258 | 0301/0604 | 1101/1305 | *0202 | 0 | 0 | 0 | 0 | 0301/1101 | 0604/1305* | 0301/1101/B3*0202 | 0604/1305/B3*0202 |
| 169 | MP259 | 0401/05032 | DR9/1501 | 0 | DR53 | 0 | 0 | 0 | 0401/0901* | 05032/1501* | 0401/0901/B4*0101(2) | 05032/1501/B5*7 |
| 170 | MP26 | 0201/0302 | 0408/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0408** | 0201/0301/B3*0101 | 0302/0408/B4*0101(2) |
| 171 | MP261 | 0501/0604 | 0101/1302 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1302 | 0501/0101/0 | 0604/1302/B3*0101 |
| 172 | MP267 | 0302/0201 | DR7/0301 | *0201 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0701(2)** | 0201/0301/B3*0201 | 0302/0701(2)/B4*0101(2) |
| 173 | MP268 | 0302/0601 | 1601/0403 | 0 | DR53 | *0102 | 0 | 0 | 0302/0403 | 0601/1601* | 0302/0403/B4*0101(2) | 0601/1601/B5*0101 |
| 174 | MP269 | 0301/0501 | 0101/0401 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0301/0401 | 0501/0101/0 | 0301/0401/B4*0101(2) |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|-------|------------|-----------|-------|------|---------|--------------|-----------|---------------|---------------|-------------------------|-------------------------|
| 175 | MP270 | 0501/0604 | 0103/1302 | *0301 | DR53 | 0 | 0 | 0 | 0501/0103 | 0604/1302 | 0501/0103/0 | 0604/1302/B3*0301 |
| 176 | MP271 | 0402/05031 | DR7/1401 | *0101 | DR53 | 0 | 0 | 0 | 05031/1401 | 0402/0701(2)* | 05031/1401/B3*0101 | 0402/0701(2)/B4*0101(2) |
| 177 | MP272 | 0201/0302 | DR7/0404 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0404 | 0201/0701(2)/B4*0101(2) | 0302/0404/B4*0101(2) |
| 178 | MP273 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 179 | MP274 | 0302/0501 | 0101/0405 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0302/0405 | 0501/0101/0 | 0302/0405/B3*0101 |
| 180 | MP275 | 0502/0603 | 0101/1302 | *0301 | 0 | 0 | 0 | 0 | 0502/0101* | 0602/1302* | 0502/0101/0 | 0603/1302/B3*0301 |
| 181 | MP278 | 0201/0302 | DR7/0407 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0407 | 0201/0701(2)/B4*0101(2) | 0302/0407/B4*0101(2) |
| 182 | MP279 | 0201/0603 | 0301/1301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1301 | 0201/0301/B3*0202 | 0603/1301/B3*0202 |
| 183 | MP28 | 0302/0603 | DR7/1101 | *0202 | DR53 | 0 | 0 | 0 | 0302/0701(2) | 0603/1101** | 0302/0701(2)/B4*0101(2) | 0603/1101/B3*0202 |
| 184 | MP280 | 0603/0604 | 1301/1301 | *0101 | 0 | 0 | 0603/1301 | 0604/1301 | 0603/1301 | 0604/1301** | 0603/1301/B3*0101 | 0604/1301/B3*0101 |
| 185 | MP281 | 0601/0601 | 1501/1501 | 0 | 0 | 0201/2 | 0601/1501 | 0601/1501 | 0601/1501** | 0601/1501** | 0601/1501/B5*0201(2) | 0601/1501/B5*0201(2) |
| 186 | MP288 | 0201/0602 | 1501/0301 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0101 | 0602/1501/B5*0101 |
| 187 | MP289 | 0402/0402 | 0801/1104 | *0202 | 0 | 0 | 0402/0801 | 0402/1104 | 0402/0801** | 0402/1104* | 0402/0801/0 | 0402/1104/B3*0202 |
| 188 | MP29 | 0301/0501 | 0101/0103 | 0 | 0 | 0 | 0 | 0 | 0301/0103** | 0501/0101 | 0301/0103/0 | 0501/0101/0 |
| 189 | MP290 | 0301/0402 | 0402/1301 | *0202 | DR53 | 0 | 0 | 0 | 0301/1301 | 0402/0402* | 0301/1301/B3*0202 | 0402/0402/B4*0101(2) |
| 190 | MP293 | 0201/0603 | 0301/1101 | *0201 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1101** | 0201/0301/B3*0201 | 0603/1101/B3*0201 |
| 191 | MP294 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 192 | MP296 | 05031/0604 | 0101/1302 | *0301 | 0 | 0 | 0 | 0 | 0604/1302 | 05031/0101* | 0604/1302/B3*0301 | 05031/0101/0 |
| 193 | MP297 | 0201/0402 | DR10/0801 | *0202 | 0 | 0 | 0 | 0 | 0201/1001* | 0402/0801 | 0201/1001/0 | 0402/0801/0 |
| 194 | MP299 | 0201/0402 | DR7/0803 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0402/0803** | 0201/0701(2)/B4*0101 | 0402/0803/0 |
| 195 | MP3 | 0201/0502 | 1501/0301 | *0101 | 0 | 0201/2 | 0 | 0 | 0201/0301 | 0502/1501 | 0201/0301/B3*0101 | 0502/1501/B5*0101(2) |
| 196 | MP30 | 0402/0605 | 0801/1401 | *0101 | 0 | 0 | 0 | 0 | 0402/0801** | 0605/1401* | 0402/0801/0 | 0605/1401/B3*0101 |
| 197 | MP300 | 0402/0201 | DR7/1201 | *0202 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0402/1201* | 0201/0701(2)/B4*0101(2) | 0402/1201/B3*0202 |
| 198 | MP32 | 0301/0601 | 0404/1501 | 0 | DR53 | *0101 | 0 | 0 | 0301/0404** | 0601/1501** | 0301/0404/B4*0101 | 0601/1501/B5*0101 |
| 199 | MP34 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 200 | MP340 | 05032/0504 | DR7/1404 | *0201 | DR53 | 0 | 0 | 0 | 05032/1404** | 0504/0701(2)* | 05032/1404/B3*0201 | 0504/0701(2)/B4*0101(2) |
| 201 | MP341 | 0201/0301 | 0301/1101 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1101 | 0201/0301/B3*0101 | 0301/1101/B3*0101 |
| 202 | MP342 | 0301/0303 | 1303/DR7 | *0101 | DR53 | 0 | 0 | 0 | 0301/1303 | 0303/0701(2) | 0301/1303/B3*0101 | 0303/0701(2)/B4*0101(2) |
| 203 | MP343 | 0302/0303 | 0402/DR9 | 0 | DR53 | 0 | 0 | 0 | 0302/0402 | 0303/0901** | 0302/0402/B4*0101 | 0303/0901/B4*0101(2) |
| 204 | MP344 | 0502/0601 | 1501/0301 | *0202 | 0 | 0201/2 | 0 | 0 | 0601/1501** | 0502/0301** | 0601/1501/B5*0201(2) | 0502/0301/B3*0202 |
| 205 | MP345 | 0201/0301 | 1103/0301 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1103 | 0201/0301/B3*0101 | 0301/1103/B3*0101 |
| 206 | MP346 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 207 | MP347 | 0201/0302 | 0404/0301 | *0201 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0404 | 0201/0301/B3*0201 | 0302/0404/B4*0101 |
| 208 | MP35 | 0603/0604 | 1301/1302 | *0201 | 0 | 0 | 0 | 0 | 0603/1301 | 0604/1302 | 0603/1301/B3*0201 | 0604/1302/B3*0201 |
| 209 | MP350 | 0201/0201 | DR10/0301 | *0101 | 0 | 0 | 0 | 0 | 0201/1001* | 0201/0301 | 0201/1001/0 | 0201/0301/B3*0101 |
| 210 | MP355 | 0301/0801 | 1102/1502 | *0301 | 0 | *0102 | 0 | 0 | 0301/1102 | 0601/1502 | 0301/1102/B4*0301 | 0601/1502/B5*0102 |
| 211 | MP356 | 0201/0201 | DR7/0301 | *0101 | DR53 | 0 | 0201/0701(2) | 0201/0301 | 0201/0701(2) | 0201/0301 | 0201/0701(2)/B4*0101 | 0201/0301/B3*0101 |
| 212 | MP357 | 0301/0301 | 0301/0801 | *0101 | 0 | 0 | 0301/0801 | 0301/0301 | 0301/0801** | 0301/0301* | 0301/0801/0 | 0301/0301/B3*0101 |
| 213 | MP358 | 0602/0401 | 0401/1302 | *0301 | DR53 | 0 | 0 | 0 | 0401/0401** | 0602/1302* | 0401/0401/B4*0101 | 0602/1302/B3*0301 |
| 214 | MP359 | 0301/0602 | 0401/1501 | 0 | DR53 | *0101 | 0 | 0 | 0301/0401 | 0602/1501 | 0301/0401/B4*0101 | 0602/1501/B5*0101 |
| 215 | MP36 | 0501/0502 | 0101/1101 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0502/1101* | 0501/0101/0 | 0502/1101/B3*0101 |
| 216 | MP360 | 0201/0201 | DR7/0301 | *0101 | DR53 | 0 | 0201/0701(2) | 0201/0301 | 0201/0701(2) | 0201/0301 | 0201/0701(2)/B4*0101 | 0201/0301/B4*0101 |
| 217 | MP361 | 0402/0601 | DR7/0302 | *0202 | 0 | 0 | 0 | 0 | 0402/0701(2)* | 0601/0302* | 0402/0701(2)/B4*0101 | 0601/0302/B3*0202 |
| 218 | MP362 | 0201/0502 | 0301/1801 | *0101 | 0 | *0201/2 | 0 | 0 | 0201/0301 | 0502/1801 | 0201/0301/B3*0101 | 0502/1801/B5*0201(2) |
| 219 | MP363 | 0501/0504 | 0101/1401 | *0201 | 0 | 0 | 0 | 0 | 0501/0101 | 0504/1401** | 0501/0101/0 | 0504/1401/B3*0201 |
| 220 | MP364 | 0201/0301 | 0401/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0301/0401 | 0201/0301/B3*0101 | 0301/0401/B4*0101 |
| 221 | MP366 | 0504/0602 | DR7/DR10 | 0 | DR53 | 0 | 0 | 0 | 0504/0701* | 0602/1001* | 0504/0701/B4*0101 | 0602/1001/0 |
| 222 | MP37 | 0201/0603 | DR9/1501 | 0 | DR53 | 0201/2 | 0 | 0 | 0201/1501* | 0603/0901* | 0201/1501/B5*0201(2) | 0603/0901/B4*0101 |
| 223 | MP372 | 0604/0605 | 1302/0401 | *0301 | DR53 | 0 | 0 | 0 | 0604/1302 | 0605/0401* | 0604/1302/B3*0301 | 0605/0401/B4*0101 |
| 224 | MP373 | 0601/0604 | 1501/1302 | *0301 | 0 | 0201/2 | 0 | 0 | 0601/1501** | 0604/1302 | 0601/1501/B5*0201(2) | 0604/1302/B3*0301 |
| 225 | MP374 | 0301/0302 | 0401/0404 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0302/0404 | 0301/0401/B4*0101 | 0302/0404/B4*0101 |
| 226 | MP375 | 0504/0602 | 1501/0302 | *0101 | 0 | 0201/2 | 0 | 0 | 0602/1501 | 0504/0302* | 0602/1501/B5*0201(2) | 0504/0302/B3*0101 |
| 227 | MP376 | 0201/0602 | 0301/1501 | *0202 | 0 | *0101 | 0 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0202 | 0602/1501/B5*0101 |
| 228 | MP378 | 0402/0501 | 0101/0401 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0402/0401* | 0501/0101/0 | 0402/0401/B4*0101(2) |
| 229 | MP379 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 230 | MP38 | 0402/0503 | 0801/1401 | *0101 | 0 | 0 | 0 | 0 | 0402/0801** | 0503/1401 | 0402/0801/0 | 0503/1401/B3*0101 |
| 231 | MP380 | 0602/0603 | 1301/1501 | *0202 | 0 | *0101 | 0 | 0 | 0602/1501 | 0603/1301 | 0602/1501/B5*0101 | 0603/1301/B3*0202 |
| 232 | MP381 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|-------|------------|------------|-------------|------|---------|--------------|--------------|---------------|----------------------|-------------------------|--------------------------|
| 233 | MP382 | 0201/0501 | DR9/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0501/0901* | 0201/0301/B3*0101 | 0501/0901/B4*0101 |
| 234 | MP383 | 05031/0604 | 1501/1302 | *0301 | 0 | 0201/2 | 0 | 0 | 05031/1501* | 0604/1302 | 05031/1501/0201(2) | 0604/1302/B3*0301 |
| 235 | MP384 | 0501/0605 | DR9/0101 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0605/0901* | 0501/0101/0 | 0605/0901/B4*0101 |
| 236 | MP386 | 0301/0604 | DR7/0407 | 0 | DR53 | 0 | 0 | 0 | 0301/0407 | 0604/0701(2)* | 0301/0407/B4*0101 | 0604/0701/B4*0101(2) |
| 237 | MP387 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 238 | MP389 | 0201/0501 | DR9/0102 | 0 | DR53 | 0 | 0 | 0 | 0501/0102 | 0201/0901* | 0501/0102/0 | 0201/0901/B4*0101 |
| 239 | MP39 | 0201/0601 | 1501/0301 | *0202 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0202 | 0601/1501/B5*0101 |
| 240 | MP391 | 0602/0603 | 1501/1301 | *0101 | 0 | *0101 | 0 | 0 | 0602/1501 | 0603/1301 | 0602/1501/B5*0101 | 0603/1301/B3*0101 |
| 241 | MP392 | 0201/0602 | 1501/0301 | *0101 | 0 | 0201/2 | 0 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0101 | 0602/1501/B5*0201(2) |
| 242 | MP393 | 0302/0303 | 0402/0402 | 0 | DR53 | 0 | 0302/0402 | 0303/0402 | 0302/0402 | 0303/0402* | 0302/0402/B4*0101(2) | 0303/0402/B4*0101(2) |
| 243 | MP394 | 0301/0302 | 0103/0410 | 0 | DR53 | 0201/2 | 0 | 0 | 0301/0103** | 0302/0410** | 0301/0103/0 | 0302/0410/B4*0101(2) |
| 244 | MP395 | 0201/0602 | DR7/1501 | 0 | DR53 | *0101 | 0 | 0 | 0201/0701(2) | 0602/1501 | 0201/0701(2)/B4*0101 | 0602/1501/B5*0101 |
| 245 | MP41 | 0302/0302 | 0401/0401 | 0 | DR53 | 0 | 0302/0401 | 0302/0401 | 0302/0401 | 0302/0401 | 0302/0401/B4*0101(2) | 0302/0401/B4*0101(2) |
| 246 | MP426 | 0602/0602 | 1501/1502 | 0 | 0 | *0201/2 | 0602/1501 | 0602/1502 | 0602/1501 | 0602/1501** | 0602/1501/B5*0201(2) | 0602/1502/B5*0201(2) |
| 247 | MP427 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 248 | MP428 | 0401/0502 | DR10/1501 | 0 | 0 | 0201/2 | 0 | 0 | 0502/1501 | 0401/1001* | 0502/1501/B5*0201(2) | 0401/1001/0 |
| 249 | MP429 | 0201/0601 | 1501/0301 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 250 | MP43 | 0301/0601 | 1201/1501 | *0202 | 0 | *0101 | 0 | 0 | 0301/1201 | 0601/1501** | 0301/1201/B3*0202 | 0601/1501/B5*0101 |
| 251 | MP430 | 0501/0501 | 0101/0101 | 0 | 0 | 0 | 0501/0101 | 0501/0101 | 0501/0101 | 0501/0101 | 0501/0101/0 | 0501/0101/0 |
| 252 | MP431 | 0201/0402 | 0801/0301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0402/0801** | 0201/0301/B3*0202 | 0402/0801/0 |
| 253 | MP432 | 05032/0603 | 1401/1104 | *0202 | 0 | 0 | 0 | 0 | 05032/1401 | 0603/1104** | 05032/1401/B3*0202 | 0603/1104/B3*0202 |
| 254 | MP433 | 0602/0603 | 1101/DR7 | *0202 | DR53 | 0 | 0 | 0 | 0603/1101** | 0602/0701(2)* | 0603/1101/B3*0202 | 0602/0701(2)/B4*0101 |
| 255 | MP434 | 0502/0602 | 1501/1601 | 0 | 0 | *0201/2 | 0 | 0 | 0502/1601 | 0602/1501 | 0502/1601/B5*0201(2) | 0602/1501/B5*0201(2) |
| 256 | MP435 | 0201/0601 | DR7/1408 | *0301 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0601/1408* | 0201/0701(2)/B4*0101 | 0601/1408/B3*0301 |
| 257 | MP438 | 0501/0501 | 0101/DR7 | 0 | DR53 | 0 | 0501/0101 | 0501/0701(2) | 0501/0101 | 0501/0701(2)* | 0501/0101/0 | 0501/0701(2)/B4*0101(2) |
| 258 | MP44 | 0201/0402 | DR9/0301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0402/0901* | 0201/0301/B3*0202 | 0402/0901/B4*0101(2) |
| 259 | MP440 | 0201/0304 | 0402/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0304/0402* | 0201/0301/B3*0101 | 0304/0402/B4*0101(2) |
| 260 | MP441 | 05031/0604 | 1302/1401 | *0101/*0301 | 0 | 0 | 0 | 0 | 05031/1401 | 0604/1302 | 05031/1401/B3*0101 | 0604/1302/B3*0301 |
| 261 | MP442 | 0501/0604 | 0101/1301 | *0202 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0202 |
| 262 | MP444 | 0302/0302 | 0402/0410 | 0 | DR53 | 0 | 0302/0402 | 0302/0410 | 0302/0402 | 0302/0410** | 0302/0402/B4*0101(2) | 0302/0410/B4*0101(2) |
| 263 | MP445 | 0501/0501 | DR7/0102 | 0 | DR53 | 0 | 0501/0102 | 0501/0701(2) | 0501/0102 | 0501/0701(2)* | 0501/0102/0 | 0501/0701(2)/B4*0101(2) |
| 264 | MP45 | 0601/0601 | DR7/1501 | 0 | DR53 | 0201/2 | 0601/1501 | 0601/0701(2) | 0601/1501** | 0601/0701(2)* | 0601/1501/B5*0201(2) | 0601/0701(2)/B4*0101(2) |
| 265 | MP46 | 0301/0603 | 0401/1302 | *0301 | DR53 | 0 | 0 | 0 | 0301/0401 | 0603/1302* | 0301/0401/B4*0101 | 0603/1302/B3*0301 |
| 266 | MP47 | 0401/0502 | 0101/1602 | 0 | 0 | *0201/2 | 0 | 0 | 0502/1602 | 0401/0101* | 0502/1602/B5*0201(2) | 0401/0101/0 |
| 267 | MP475 | 0201/0302 | DR7/0401 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0401 | 0201/0701/B4*0101(2) | 0302/0401/B4*0101(2) |
| 268 | MP476 | 05032/0601 | 1501/DR7 | 0 | DR53 | *0101 | 0 | 0 | 0601/1501** | 05032/1701(2)* | 0601/1501/B5*0101 | 05032/0701/B4*0101 |
| 269 | MP477 | 0501/0604 | 0101/1402 | *0301 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1402* | 0501/0101/0 | 0604/1402/B3*0301 |
| 270 | MP478 | 0302/0402 | 0801/08042 | *0101 | 0 | 0 | 0 | 0 | 0302/08041** | 0402/0801* | 0302/08042/0 | 0402/0801/0 |
| 271 | MP48 | 0301/0301 | 0103/DR10 | 0 | 0 | 0 | 0301/0103 | 0301/1001 | 0301/0103** | 0301/1001* | 0301/0103/0 | 0301/1001/0 |
| 272 | MP480 | 0603/0604 | 1101/1302 | *0301/0202 | 0 | 0 | 0 | 0 | 0603/1101** | 0604/1302 | 0603/1101/B3*0202 | 0604/1302/B3*0301 |
| 273 | MP481 | 0301/0302 | 0401/0404 | 0 | DR53 | 0 | 0 | 0 | 0301/0401 | 0302/0404 | 0301/0401/B4*0101(2) | 0302/0404/B4*0101(2) |
| 274 | MP482 | 0502/0604 | 1601/1301 | *0101 | 0 | *0201/2 | 0 | 0 | 0502/1601 | 0604/1301** | 0502/1601/B5*0201(2) | 0604/1301/B3*0101 |
| 275 | MP483 | 0201/0303 | 1301/0301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0303/1301 | 0201/0301/B3*0202 | 0303/1301/B3*0202 |
| 276 | MP484 | 0201/0601 | 1501/0301 | *0202 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0202 | 0601/1501/B5*0101 |
| 277 | MP486 | 0602/0603 | DR7/DR9 | 0 | DR53 | 0 | 0 | 0 | 0602/0701(2)* | 0603/0901* | 0602/0701(2)/B4*0101(2) | 0603/0901/B4*0101 |
| 278 | MP487 | 0501/0605 | 0101/0301 | *0202 | 0 | 0 | 0 | 0 | 0501/0101 | 0605/0301* | 0501/0101/0 | 0605/0301/B3*0202 |
| 279 | MP488 | 05031/0602 | 1401/DR7 | *0101 | DR53 | 0 | 0 | 0 | 05031/1401 | 0602/0701(2) | 05031/1401/B3*0101 | 0602/0701(2)/B4*0101(2) |
| 280 | MP489 | 0301/0601 | 0401/1502 | 0 | DR53 | *0102 | 0 | 0 | 0601/1502 | 0301/0401 | 0601/1502/B5*0102 | 0301/0401/B4*0101 |
| 281 | MP49 | 0201/0601 | 0301/DR9 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0601/0901* | 0201/0301/B3*0202 | 0601/0901/B4*0101 |
| 282 | MP491 | 0402/05032 | DR7/0803 | 0 | DR53 | 0 | 0 | 0 | 0402/0803** | 05032/0701(2)* | 0402/0803/0 | 05032/0701(2)/B4*0101(2) |
| 283 | MP492 | 0201/0201 | DR7/DR7 | 0 | DR53 | 0 | 0201/0701(2) | 0201/0701(2) | 0201/0701(2) | 0201/0701(2) | 0201/0701(2)/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 284 | MP493 | 0501/0504 | DR9/0101 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0504/0901* | 0501/0101/0 | 0504/0901/B4*0101 |
| 285 | MP494 | 0302/0302 | DR7/0401 | 0 | DR53 | 0 | 0302/0401 | 0302/0701(2) | 0302/0401 | 0302/0701(2)/B4*0101 | 0302/0401/B4*0101(2) | 0302/0401/B4*0101(2) |
| 286 | MP495 | 0402/0605 | 0801/1301 | *0202 | 0 | 0 | 0 | 0 | 0402/0801** | 0605/1301* | 0402/0801/0 | 0605/1301/B3*0202 |
| 287 | MP496 | 0302/0302 | 0404/1101 | *0202 | DR53 | 0 | 0302/0404 | 0302/1101 | 0302/0404 | 0302/1101 | 0302/0404/B4*0101 | 0302/1101/B3*0202 |
| 288 | MP497 | 0605/0605 | 1301/0404 | *0101 | DR53 | 0 | 0605/1301 | 0605/0404 | 0605/1301* | 0605/0404* | 0605/1301/B3*0101 | 0605/0404/B4*0101(2) |
| 289 | MP498 | 0302/0303 | 0404/1301 | *0101 | DR53 | 0 | 0 | 0 | 0302/0404 | 0303/1301 | 0302/0404/B4*0101(2) | 0303/1301/B3*0101 |
| 290 | MP499 | 0504/0601 | 1104/1502 | *0202 | 0 | *0102 | 0 | 0 | 0601/1502 | 0504/1104* | 0601/1502/B5*0102 | 0504/1104/B3*0202 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|-------|------------|------------|-------|------|---------|--------------|--------------|----------------|----------------|-------------------------|-------------------------|
| 291 | MP50 | 0302/0302 | 0401/0402 | 0 | DR53 | 0 | 0302/0401 | 0302/0402 | 0302/0401 | 0302/0402 | 0302/0401/B4*0101(2) | 0302/0402/B4*0101(2) |
| 292 | MP500 | 0401/0402 | 0401/0803 | *0101 | DR53 | 0 | 0 | 0 | 0401/0401** | 0402/0803** | 0401/0401/B4*0101(2) | 0402/0803/0 |
| 293 | MP501 | 0502/0604 | 1601/1301 | *0101 | 0 | *0201/2 | 0 | 0 | 0502/1601 | 0604/1301** | 0502/1601/B5*0201(2) | 0604/1301/B3*0101 |
| 294 | MP507 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 295 | MP502 | 0303/0303 | DR9/DR9 | 0 | DR53 | 0 | 0303/0901 | 0303/0901 | 0303/0901** | 0303/0901** | 0303/0901/B4*0101(2) | 0303/0901/B4*0101(2) |
| 296 | MP503 | 0201/0604 | DR7/1301 | *0201 | 0 | 0 | 0 | 0 | 0201/0701(2) | 0604/1301** | 0201/0701(2)/B4*0101(2) | 0604/1301/B3*0201 |
| 297 | MP506 | 0402/0501 | DR7/0101 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0402/0701(2)* | 0501/0101/0 | 0402/0701(2)/B4*0101(2) |
| 298 | MP52 | 0201/0604 | DR9/1301 | *0202 | DR53 | 0 | 0 | 0 | 0604/1301** | 0201/0901* | 0604/1301/B3*0202 | 0201/0901/B4*0101(2) |
| 299 | MP54 | 0603/0604 | 1104/1301 | *0202 | 0 | 0 | 0 | 0 | 0603/1104** | 0604/1301** | 0603/1104/B3*0202 | 0604/1301/B3*0202 |
| 300 | MP55 | 0302/0603 | 0402/1101 | *0202 | DR53 | 0 | 0 | 0 | 0603/1101** | 0302/0402 | 0603/1101/B3*0202 | 0302/1402/B4*0101(2) |
| 301 | MP56 | 0501/0604 | DR9/1301 | *0202 | DR53 | 0 | 0 | 0 | 0604/1301** | 0501/0901* | 0604/1301/B3*0202 | 0501/0901/B4*0101(2) |
| 302 | MP57 | 0301/0504 | 0103/1404 | *0202 | 0 | 0 | 0 | 0 | 0301/0103** | 0504/1104** | 0301/0103/0 | 0504/1404/B3*0202 |
| 303 | MP58 | 0201/0201 | DR7/0301 | *0202 | DR53 | 0 | 0201/0301 | 0201/0701(2) | 0201/0301 | 0201/0701(2) | 0201/0301/B3*0202 | 0201/0701(2)/B4*0101(2) |
| 304 | MP59 | 0302/05031 | 0401/1401 | *0101 | DR53 | 0 | 0 | 0 | 0302/0401 | 05032/1401 | 0302/0401/B4*0101(2) | 05032/1401/B3*0101 |
| 305 | MP6 | 0201/0601 | 1407/0301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0601/1407** | 0201/0301/B3*0202 | 0601/1407/B3*0202 |
| 306 | MP60 | 0301/0502 | 1301/1601 | *0202 | 0 | 0201/2 | 0 | 0 | 0301/1301 | 0502/1601 | 0301/1301/B3*0202 | 0502/1601/B5*0201(2) |
| 307 | MP61 | 0201/0301 | 0301/1101 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/1101 | 0201/0301/B3*0101 | 0301/1101/B3*0101 |
| 308 | MP62 | 0502/0604 | 1601/1301 | *0101 | 0 | *0102 | 0 | 0 | 0502/1601 | 0604/1301** | 0502/1601/B5*0102 | 0604/1301/B3*0101 |
| 309 | MP65 | 0201/0201 | DR9/0301 | *0101 | DR53 | 0 | 0201/0301 | 0201/0901 | 0201/0301 | 0201/0901* | 0201/0301/B3*0101 | 0201/0901/B4*0101(2) |
| 310 | MP66 | 0301/0402 | 1301/0801 | *0101 | 0 | 0 | 0 | 0 | 0301/1301 | 0402/0801** | 0301/1301/B3*0101 | 0402/0801/0 |
| 311 | MP67 | 0201/0501 | 0101/0301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0501/0101 | 0201/0301/B3*0202 | 0501/0101/0 |
| 312 | MP687 | 0501/0502 | 0101/1501 | 0 | 0 | 0201/2 | 0 | 0 | 0501/0101 | 0502/1501 | 0501/0101/0 | 0502/1501/B5*0201(2) |
| 313 | MP689 | 0501/0603 | DR7/0102 | 0 | DR53 | 0 | 0 | 0 | 0501/0102 | 0603/0701(2)* | 0501/0102/0 | 0603/0701(2)/B4*0101(2) |
| 314 | MP69 | 0302/0602 | 0401/08031 | 0 | DR53 | 0 | 0 | 0 | 0302/0401 | 0602/08031* | 0302/0401/B4*0101(2) | 0602/0803/0 |
| 315 | MP691 | 0201/0201 | DR7/DR7 | 0 | DR53 | 0 | 0201/0701(2) | 0201/0701(2) | 0201/0701(2) | 0201/0701(2) | 0201/0701(2)/B4*0101(2) | 0201/0701(2)/B4*0101(2) |
| 316 | MP692 | 0604/0605 | DR7/1301 | *0101 | DR53 | 0 | 0 | 0 | 0604/1301** | 0605/0701(2)* | 0604/1301/B3*0101 | 0605/0701(2)/B4*0101(2) |
| 317 | MP693 | 0201/0502 | 1601/0301 | *0202 | 0 | *0201/2 | 0 | 0 | 0201/0301 | 0502/1601 | 0201/0301/B3*0202 | 0502/1601/B5*0201(2) |
| 318 | MP695 | 0201/0604 | 0301/1301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1301** | 0201/0301/B3*0202 | 0604/1301/B3*0202 |
| 319 | MP697 | 0201/0602 | 0301/1501 | *0202 | 0 | *0101 | 0 | 0 | 0201/0301 | 0602/1501 | 0201/0301/B3*0202 | 0602/1501/B5*0101 |
| 320 | MP698 | 0302/0603 | 0401/1101 | *0202 | DR53 | 0 | 0 | 0 | 0302/0401 | 0603/1101** | 0302/0401/B4*0101 | 0603/1101/B3*0202 |
| 321 | MP699 | 0501/0604 | 0101/1301 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/1301** | 0501/0101/0 | 0604/1301/B3*0101 |
| 322 | MP7 | 0502/0603 | 0302/1303 | *0202 | 0 | 0 | 0 | 0 | 0502/0302* | 0603/1303* | 0502/0302/B3*0202 | 0603/1303/B3*0202 |
| 323 | MP70 | 0201/0201 | 0408/0301 | *0101 | DR53 | 0 | 0201/0408 | 0201/0301 | 0201/0408* | 0201/0301 | 0201/0408/B4*0101(2) | 0201/0301/B3*0101 |
| 324 | MP701 | 0301/0301 | 1104/1305 | *0101 | 0 | 0 | 0301/1104 | 0301/1305 | 0301/1104 | 0301/1305 | 0301/1104/B3*0101 | 0301/1305/B3*0101 |
| 325 | MP702 | 0501/0501 | DR7/0101 | 0 | DR53 | 0 | 0501/0101 | 0501/0701(2) | 0501/0101 | 0501/0701(2)* | 0501/0101/0 | 0501/0701(2)/B4*0101(2) |
| 326 | MP703 | 0302/0302 | DR7/0801 | 0 | DR53 | 0 | 0302/0701(2) | 0302/0801 | 0302/0701(2)** | 0302/0801* | 0302/0701(2)/B4*0101(2) | 0302/0801/0 |
| 327 | MP704 | 0201/0605 | DR9/0301 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0605/0901* | 0201/0301/B3*0202 | 0605/0901/B4*0101(2) |
| 328 | MP753 | 0201/0601 | 0301/1502 | *0101 | 0 | *0102 | 0 | 0 | 0201/0301 | 0601/1502 | 0201/0301/B3*0101 | 0601/1502/B5*0102 |
| 329 | MP754 | 0602/0604 | DR9/1501 | 0 | DR53 | *0101 | 0 | 0 | 0602/1501 | 0604/0901* | 0602/1501/B5*0101 | 0604/0901/B4*0101(2) |
| 330 | MP756 | 0401/0502 | DR7/1501 | 0 | DR53 | 0201/2 | 0 | 0 | 0401/1501* | 0502/1701(2)* | 0401/1501/B5*0201(2) | 0502/0701(2)/B4*0101(2) |
| 331 | MP758 | 0201/0302 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0401 | 0201/0301/B3*0101 | 0302/0401/B4*0101(2) |
| 332 | MP760 | 0501/0605 | 0101/1301 | *0201 | 0 | 0 | 0 | 0 | 0501/0101 | 0605/1301 | 0501/0101/0 | 0605/1301/B3*0201 |
| 333 | MP762 | 0603/0604 | DR9/1301 | *0101 | DR53 | 0 | 0 | 0 | 0603/1301 | 0604/0901* | 0603/1301/B3*0101 | 0604/0901/B4*0101 |
| 334 | MP763 | 0201/0601 | 0301/1501 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 335 | MP766 | 0201/0602 | DR9/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0602/0901* | 0201/0301/B3*0101 | 0602/0901/B4*0101 |
| 336 | MP767 | 0302/0201 | 0402/0301 | *0101 | DR53 | 0 | 0 | 0 | 0302/0402 | 0201/0301 | 0302/0402/B4*0101 | 0201/0301/B3*0101 |
| 337 | MP768 | 0402/05031 | DR7/0302 | *0202 | DR53 | 0 | 0 | 0 | 0402/0701(2)* | 05031/0302* | 0402/0701(2)/B4*0101(2) | 05031/0302/B3*0202 |
| 338 | MP770 | 0301/0301 | 0401/0103 | 0 | DR53 | 0 | 0301/0401 | 0301/0103 | 0301/0401 | 0301/0103** | 0301/0401/B4*0101(2) | 0301/0103/0 |
| 339 | MP771 | 0201/0502 | 0301/1602 | *0101 | 0 | *0201/2 | 0 | 0 | 0201/0301 | 0502/1602** | 0201/0301/B3*0101 | 0502/1602/B5*0201(2) |
| 340 | MP772 | 0302/0401 | DR7/0401 | 0 | DR53 | 0 | 0 | 0 | 0302/0401 | 0401/0401** | 0302/0401/B4*0101(2) | 0401/0401/B4*0101(2) |
| 341 | MP773 | 0201/0604 | DR9/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0604/0901* | 0201/0301/B3*0101 | 0604/0901/B4*0101 |
| 342 | MP774 | 0201/0302 | DR7/0301 | *0202 | DR53 | 0 | 0 | 0 | 0201/0301 | 0302/0701(2)** | 0201/0301/B3*0202 | 0302/0701(2)/B4*0101 |
| 343 | MP775 | 05031/0601 | 1501/1401 | *0101 | 0 | *0101 | 0 | 0 | 0601/1501** | 05031/1401 | 0601/1501/B5*0101 | 05031/1401/B3*0101 |
| 344 | MP777 | 0501/0501 | DR9/0101 | 0 | DR53 | 0 | 0501/0101 | 0501/0901 | 0501/0101 | 0501/0901* | 0501/0101/0 | 0501/0901/B4*0101(2) |
| 345 | MP778 | 0201/0301 | 0301/0401 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0301/0401 | 0201/0301/B3*0101 | 0301/0401/B4*0101 |
| 346 | MP780 | 0402/0501 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 347 | MP781 | 0201/0302 | DR7/0408 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0408** | 0201/0701/B4*0101 | 0302/0408/B4*0101(2) |
| 348 | MP782 | 0303/0303 | 1301/1301 | *0101 | 0 | 0 | 0303/1301 | 0303/1301 | 0303/1301 | 0303/1301 | 0303/1301/B3*0101 | 0303/1301/B3*0101 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|-------|------------|-----------|------------|------|---------|--------------|---------------|---------------|----------------|-------------------------|-------------------------|
| 349 | MP79 | 0201/0301 | 0401/0302 | *0202 | DR53 | 0 | 0 | 0 | 0201/0302* | 0301/0401 | 0201/0302/B3*0202 | 0301/0401/B4*0101 |
| 350 | MP799 | 0201/0604 | 0301/1407 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1407* | 0201/0301/B3*0101 | 0604/1407/B3*0101 |
| 351 | MP8 | 0502/0603 | DR10/1101 | *0202 | 0 | 0 | 0 | 0 | 0502/1001* | 0603/1101** | 0502/1001/0 | 0603/1101/B3*0202 |
| 352 | MP80 | 0201/0601 | 0301/1501 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/* |
| 353 | MP800 | 0401/0402 | DR7/0401 | 0 | DR53 | 0 | 0 | 0 | 0401/0401 | 0402/0701(2)* | 0401/0401/B4*0101(2) | 0402/0701(2)/B4*0101(2) |
| 354 | MP801 | 0201/0602 | DR7/1502 | 0 | DR53 | *0102 | 0 | 0 | 0201/0701 | 0602/1502** | 0201/0701/B4*0101(2) | 0602/1502/B5*0102 |
| 355 | MP802 | 0401/0501 | DR7/0101 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0401/0701(2)* | 0501/0101/0 | 0401/0701(2)/B4*0101(2) |
| 356 | MP803 | 0302/0302 | DR10/0404 | 0 | DR53 | 0 | 0302/0404 | 0302/1001 | 0302/0404 | 0302/1001* | 0302/0404/B4*0101(2) | 0302/1001/0 |
| 357 | MP804 | 0502/05031 | 1601/1401 | *0101 | 0 | 0 | 0 | 0 | 0502/1601 | 05031/1401 | 0502/1601/* | 05031/1401/B3*0101 |
| 358 | MP805 | 0201/0301 | DR7/0103 | 0 | DR53 | 0 | 0 | 0 | 0201/0701 | 0301/0103** | 0201/0701(2)/B4*0101(2) | 0301/0103/0 |
| 359 | MP806 | 0501/0604 | DR7/0101 | *0101 | 0 | 0 | 0 | 0 | 0501/0101 | 0604/0701(2)* | 0501/0101/0 | 0604/0701(2)/0 |
| 360 | MP807 | 0201/0301 | DR7/0408 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0301/0408 | 0201/0701(2)/B4*0101(2) | 0301/0408/B4*0101(2) |
| 361 | MP808 | 0201/0601 | 0301/1501 | *0101 | 0 | *0101 | 0 | 0 | 0201/0301 | 0601/1501** | 0201/0301/B3*0101 | 0601/1501/B5*0101 |
| 362 | MP809 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 363 | MP81 | 0504/0603 | 1104/1305 | 0301/0201 | 0 | 0 | 0 | 0 | 0603/1104** | 0504/1305* | 0603/1104/B3*0201 | 0504/1305/B3*0301 |
| 364 | MP811 | 0401/0603 | 1101/0401 | *0202 | DR53 | 0 | 0 | 0 | 0401/1401** | 0603/1101** | 0401/0401/B4*0101 | 0603/1101/B3*0202 |
| 365 | MP812 | 0201/0302 | DR7/0401 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0302/0401 | 0201/0701(2)/B4*0101(2) | 0302/0401/B4*0101(2) |
| 366 | MP813 | 0302/0302 | 0402/0403 | 0 | DR53 | 0 | 0302/0402 | 0302/0403 | 0302/0402 | 0302/0403 | 0302/0402/B4*0101 | 0302/0403/B4*0101(2) |
| 367 | MP814 | 0501/0604 | 0102/1302 | *0301 | 0 | 0 | 0 | 0 | 0501/0102 | 0604/1302 | 0501/0102/0 | 0604/1302/B3*0301 |
| 368 | MP815 | 0201/0603 | 1301/0301 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0603/1301 | 0201/0301/B3*0202 | 0603/1301/B3*0202 |
| 369 | MP816 | 0402/0501 | 0101/0801 | 0 | 0 | 0 | 0 | 0 | 0501/0101 | 0402/0801** | 0501/0101/0 | 0402/0801/0 |
| 370 | MP817 | 0201/0604 | 0301/1301 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1301** | 0201/0301/B3*0101 | 0604/1301/B3*0101 |
| 371 | MP818 | 0201/0604 | DR7/0301 | *0201 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/0701(2)* | 0201/0301/B3*0201 | 0604/0701(2)/B4*0101(2) |
| 372 | MP819 | 0201/0604 | 0301/1302 | *0101/0301 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1302 | 0201/0301/B3*0101 | 0604/1302/B3*0301 |
| 373 | MP82 | 05032/0601 | 0102/1501 | 0 | 0 | 0201/2 | 0 | 0 | 0601/1501** | 05032/0102* | 0601/1501/B5*0201(2) | 05032/0102/0 |
| 374 | MP820 | 0501/05031 | DR10/1403 | *0301 | 0 | 0 | 0 | 0 | 05031/1403** | 0501/1001 | 05031/1403/B3*0301 | 0501/1001/0 |
| 375 | MP821 | 0302/0303 | 0401/1301 | 0 | DR53 | 0 | 0 | 0 | 0302/0401 | 0303/1301 | 0302/0401/B4*0101(2) | 0303/1301/B3* |
| 376 | MP822 | 0301/0201 | DR9/0301 | *0101 | DR53 | 0 | 0 | 0 | 0301/0901* | 0201/0301 | 0301/0901/B4*0101(2) | 0201/0301/B3*0101 |
| 377 | MP828 | 0303/0501 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 378 | MP823 | 0201/0604 | DR7/DR7 | 0 | DR53 | 0 | 0201/0701(2) | 0604/0701(2) | 0201/0701(2) | 0604/0701(2)* | 0201/0701(2)/B4*0101(2) | 0604/0701/B4*0101(2) |
| 379 | MP824 | 0603/0604 | DR7/1301 | *0101 | DR53 | 0 | 0 | 0 | 0603/1301 | 0604/0701(2)* | 0603/1301/B3*0101 | 0604/0701(2)/B4*0101(2) |
| 380 | MP825 | 0301/0301 | 0103/0401 | 0 | DR53 | 0 | 0301/0103 | 0301/0401 | 0301/0103** | 0301/0401 | 0301/0103/0 | 0301/0401/B4*0101(2) |
| 381 | MP826 | 0501/0502 | DR7/1601 | 0 | DR53 | *0201/2 | 0 | 0 | 0502/1601 | 0501/0701(2)* | 0502/1601/B5*0201(2) | 0501/0701/B4*0101(2) |
| 382 | MP827 | 0301/0301 | DR9/1104 | *0101 | DR53 | 0 | 0301/0901 | 0301/1104 | 0301/0901* | 0301/1104 | 0301/0901/B4*0101(2) | 0301/1104/B3*0101 |
| 383 | MP83 | 0301/0603 | 1101/0403 | *0202 | DR53 | 0 | 0 | 0 | 0301/0403** | 0603/1101** | 0301/0403/B4*0101(2) | 0603/1101/B3*0202 |
| 384 | MP84 | 0201/0303 | DR7/1301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0701 | 0303/1301 | 0201/0701/B4*0101(2) | 0303/1301/B3*0101 |
| 385 | MP85 | 0601/0605 | 1501/1501 | 0 | 0 | 0201/2 | 0601/1501 | 0605/1501 | 0601/1501** | 0605/1501* | 0601/1501/B5*0201(2) | 0605/1501/B5*0201(2) |
| 386 | MP86 | 0201/0201 | 0301/0301 | *0202 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301 | 0201/0301/B3*0202 | 0201/0301/B3*0202 |
| 387 | MP87 | 0502/0603 | 1602/1104 | *0202 | 0 | *0201/2 | 0 | 0 | 0502/1602** | 0603/1104** | 0502/1602/B5*0201(2) | 0603/1104/B3*0202 |
| 388 | MP88 | 0602/0604 | 1501/1401 | *0101 | 0 | 0 | 0 | 0 | 0602/1501 | 0604/1401* | 0602/1501/B5* | 0604/1401/B3*0101 |
| 389 | MP89 | 0201/0501 | DR10/0101 | 0 | 0 | 0 | 0 | 0 | 0501/0101 | 0201/1001* | 0501/0101/0 | 0201/1001/0 |
| 390 | MP9 | 0604/0604 | 1301/1302 | *0201/0202 | 0 | 0 | 0604/1301 | 0604/1302 | 0604/1301** | 0604/1302 | 0604/1301/B3*0201 | 0604/1302/B3*0202 |
| 391 | MP90 | 0301/0302 | DR7/1101 | *0202 | DR53 | 0 | 0 | 0 | 0301/1101 | 0302/0701(2)** | 0301/1101/B3*0202 | 0302/0701(2)/B4*0101(2) |
| 392 | MP91 | 0603/0604 | 0302/1302 | *0101 | 0 | 0 | 0 | 0 | 0603/0302* | 0604/1302 | 0603/0302/B3*0101 | 0604/1302/B3*0101 |
| 393 | MP92 | 0502/0602 | DR10/1501 | 0 | 0 | *0101 | 0 | 0 | 0502/1501 | 0602/1001* | 0502/1501/B5*0101 | 0602/1001/0 |
| 394 | MP93 | 0201/0301 | DR7/0401 | 0 | DR53 | 0 | 0 | 0 | 0201/0701(2) | 0301/0401 | 0201/0701(2)/B4*0101(2) | 0301/0401/B4*0101(2) |
| 395 | MP934 | 0501/0501 | DR7/0102 | 0 | DR53 | 0 | 0501/0102 | 0501/0701(2)* | 0501/0102 | 0501/0701(2)* | 0501/0102/0 | 0501/0701(2)/B4*0101(2) |
| 396 | MP935 | 0302/0302 | 0401/0801 | 0 | DR53 | 0 | 0302/0401 | 0302/0801 | 0302/0401 | 0302/0801* | 0302/0401/B4*0101 | 0302/0801/0 |
| 397 | MP936 | 05031/0603 | DR7/1401 | *0202 | DR53 | 0 | 0 | 0 | 05031/1401 | 0603/0701(2)* | 05031/1401/B3*0202 | 0603/0701(2)/B4*0101(2) |
| 398 | MP937 | 0501/0604 | 0102/1302 | *0301 | 0 | 0 | 0 | 0 | 0501/0102 | 0604/1302 | 0501/0102/0 | 0604/1302/B3*0301 |
| 399 | MP938 | 0402/0402 | 0801/1302 | *0301 | 0 | 0 | 0402/0801 | 0402/1302 | 0402/1302** | 0402/0801* | 0402/1302/B3*0101 | 0402/1302/B3*0301 |
| 400 | MP939 | 0201/0601 | 0408/0301 | *0101 | DR53 | 0 | 0 | 0 | 0201/0301 | 0601/0408* | 0201/0301/B3*0101 | 0601/0408/B4*0101(2) |
| 401 | MP94 | 0302/0303 | 0404/1301 | *0101 | DR53 | 0 | 0 | 0 | 0302/0404 | 0303/1301 | 0302/0404/B4*0101 | 0303/1301/B3*0101 |
| 402 | MP940 | 0501/0501 | 0101/0101 | 0 | 0 | 0 | 0501/0101 | 0501/0101 | 0501/0101 | 0501/0101 | 0501/0101/0 | 0501/0101/0 |
| 403 | MP941 | 0603/0604 | 1101/1301 | *0101 | 0 | 0 | 0 | 0 | 0603/1101** | 0604/1301** | 0603/1101/B3*0101 | 0604/1301/B3*0101 |
| 404 | MP942 | 0605/0605 | DR7/0301 | *0202 | DR53 | 0 | 0605/0701(2) | 0605/0301 | 0605/0701(2)* | 0605/0301* | 0605/0701/B4*0101(2) | 0605/0301/B3*0202 |
| 405 | MP943 | 0401/0502 | DR9/0410 | 0 | DR53 | 0 | 0 | 0 | 0401/0410* | 0502/0901* | 0401/0410/B4*0101(2) | 0502/0901/B4*0101(2) |
| 406 | MP944 | 0201/0301 | 0101/0301 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0301/0101* | 0201/0301/B3*0101 | 0301/0101/0 |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|-------|------------|-----------|------------|------|---------|-----------|-----------|---------------|---------------|-------------------------|-------------------------|
| 407 | MP946 | 0402/05031 | 0803/1401 | *0101 | 0 | 0 | 0 | 0 | 0402/0803** | 05031/1401 | 0402/0803/0 | 05031/1401/B3*0101 |
| 408 | MP947 | 0501/0502 | DR9/0101 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0502/0901* | 0501/0101/0 | 0502/0901/B4*0101(2) |
| 409 | MP948 | 0201/0601 | 0301/1502 | *0202 | 0 | *0102 | 0 | 0 | 0201/0301 | 0601/1502 | 0201/0301/B3*0202 | 0601/1502/B5*0102 |
| 410 | MP949 | 0201/0201 | 0301/0301 | *0101 | 0 | 0 | 0 | 0 | 0201/0301 | 0201/0301 | 0201/0301/B3*0101 | 0201/0301/B3*0101 |
| 411 | MP95 | 0302/05032 | 1501/1402 | *0101 | 0 | *0101 | 0 | 0 | 0302/1501* | 05032/1402 | 0302/1501/B5*0101 | 05032/1402/B3*0101 |
| 412 | MP950 | 0402/0602 | DR7/1502 | 0 | DR53 | *0102 | 0 | 0 | 0602/1502** | 0402/0701* | 0602/1502/B5*0102 | 0402/0701/B4*0101 |
| 413 | MP951 | 0602/0201 | 1501/DR7 | 0 | DR53 | *0101 | 0 | 0 | 0201/0701(2) | 0602/1501 | 0201/0701(2)/B4*0101(2) | 0602/1501/B5*0101 |
| 414 | MP952 | 0501/0604 | DR7/DR7 | 0 | DR53 | 0 | 0501/0701 | 0604/0701 | 0501/0701(2)* | 0604/0701(2)* | 0501/0701/B4*0101(2) | 0604/0701(2)/B4*0101(2) |
| 415 | MP953 | 0502/0603 | 1301/1601 | *0101 | 0 | *0201/2 | 0 | 0 | 0502/1601 | 0603/1301 | 0502/1601/B5*0201(2) | 0603/1301/B3*0101 |
| 416 | MP954 | 0201/0602 | DR7/1502 | 0 | DR53 | *0102 | 0 | 0 | 0201/0701(2) | 0602/1502** | 0201/0701(2)/B4*0101(2) | 0602/1502/B5*0102 |
| 417 | MP955 | 0402/05031 | DR9/1401 | *0201 | DR53 | 0 | 0 | 0 | 05031/1401 | 0402/0901* | 0503/1401/B3*0201 | 0402/0901/B4*0101(2) |
| 418 | MP956 | 0201/0604 | 0301/1302 | *0301/0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0604/1302 | 0201/0301/B3*0202 | 0604/1302/B3*0301 |
| 419 | MP96 | 0603/0604 | 1101/1301 | *0202 | 0 | 0 | 0 | 0 | 0603/1101** | 0604/1301** | 0603/1101/B3*0202 | 0604/1301/B3*0202 |
| 420 | MP97 | 0501/0601 | DR9/0101 | 0 | DR53 | 0 | 0 | 0 | 0501/0101 | 0601/0901* | 0501/0101/0 | 0601/0901/B4*0101 |
| 421 | MP98 | 0201/0501 | 0301/0101 | *0202 | 0 | 0 | 0 | 0 | 0201/0301 | 0501/0101 | 0201/0301/B3*0202 | 0501/0101/0 |
| 422 | | | | | | | | | | | | |

**APPENDIX III: FULL MOTIF PREDICTION FROM HPV 16 E6, E7, L1
AND L2 FOR BINDING TO HLA DRB1*0101 AND DRB1*0401**

| | | | | | | | | | | | | | | | | IC50 DRB1*0101(nm) |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--|--------------------|
| 59 | 71 | I | V | Y | R | D | G | N | P | Y | A | V | C | D | | 4 |
| 33 | 45 | I | I | L | E | C | V | Y | C | K | Q | Q | L | L | | 12 |
| 142 | 154 | R | C | M | S | C | C | R | S | S | R | T | R | R | | 12.5 |
| 75 | 87 | K | F | Y | S | K | I | S | E | Y | R | H | Y | C | | 18.2 |
| 84 | 96 | R | H | Y | C | Y | S | L | Y | G | T | T | L | E | | 34.5 |
| 130 | 142 | Q | R | F | H | N | I | R | G | R | W | T | G | R | | 56.2 |
| 106 | 118 | L | L | I | R | C | I | N | C | Q | K | P | L | C | | 170 |
| 101 | 113 | K | P | L | C | D | L | L | I | R | C | I | N | C | | 185 |
| 93 | 105 | T | T | L | E | Q | Q | Y | N | K | P | L | C | D | | 265 |
| 133 | 145 | H | N | I | R | G | R | W | T | G | R | C | M | S | | 362 |
| 109 | 121 | R | C | I | N | C | Q | K | P | L | C | P | E | E | | 365 |
| 36 | 48 | E | C | V | Y | C | K | Q | Q | L | L | R | R | E | | 415 |
| 74 | 86 | L | K | F | Y | S | K | I | S | E | Y | R | H | Y | | 440 |
| 50 | 62 | Y | D | F | A | F | R | D | L | C | I | V | Y | R | | 520 |
| 37 | 49 | C | V | Y | C | K | Q | Q | L | L | R | R | E | V | | 622 |
| 105 | 117 | D | L | L | I | R | C | I | N | C | Q | K | P | L | | 770 |
| 52 | 64 | F | A | F | R | D | L | C | I | V | Y | R | D | G | | 825 |
| 81 | 93 | S | E | Y | R | H | Y | C | Y | S | L | Y | G | T | | 880 |
| 88 | 100 | Y | S | L | Y | G | T | T | L | E | Q | Q | Y | N | | 1300 |
| 31 | 43 | H | D | I | I | L | E | C | V | Y | C | K | Q | Q | | 1900 |
| 58 | 70 | C | I | V | Y | R | D | G | N | P | Y | A | V | C | | 2600 |
| 28 | 40 | T | T | I | H | D | I | I | L | E | C | V | Y | C | | 3200 |
| 48 | 60 | E | V | Y | D | F | A | F | R | D | L | C | I | V | | 3350 |
| 57 | 69 | L | C | I | V | Y | R | D | G | N | P | Y | A | V | | 4000 |
| 24 | 36 | T | E | L | Q | T | T | I | H | D | I | I | L | E | | 4600 |
| 80 | 92 | I | S | E | Y | R | H | Y | C | Y | S | L | Y | G | | 5900 |
| 47 | 59 | R | E | V | Y | D | F | A | F | R | D | L | C | I | | 6120 |
| 97 | 109 | Q | Q | Y | N | K | P | L | C | D | L | L | I | R | | 6500 |
| 32 | 44 | D | I | I | L | E | C | V | Y | C | K | Q | Q | L | | 7000 |
| 127 | 139 | D | K | K | Q | R | F | H | N | I | R | G | R | W | | 7350 |
| 65 | 77 | N | P | Y | A | V | C | D | K | C | L | K | F | Y | | 10200 |
| 72 | 84 | K | C | L | K | F | Y | S | K | I | S | E | Y | R | | 11000 |
| 78 | 90 | S | K | I | S | E | Y | R | H | Y | C | Y | S | L | | 11000 |
| 137 | 149 | G | R | W | T | G | R | C | M | S | C | C | R | S | | 12000 |
| 43 | 55 | Q | L | L | R | R | E | V | Y | D | F | A | F | R | | 29200 |
| 67 | 79 | Y | A | V | C | D | K | C | L | K | F | Y | S | K | | 35000 |
| 71 | 83 | D | K | C | L | K | F | Y | S | K | I | S | E | Y | | 37000 |
| 6 | 18 | T | A | M | F | Q | D | P | Q | E | R | P | R | K | | 39500 |
| 140 | 152 | T | G | R | C | M | S | C | C | R | S | S | R | T | | 45000 |
| 104 | 116 | C | D | L | L | I | R | C | I | N | C | Q | K | P | | 60000 |
| 139 | 151 | W | T | G | R | C | M | S | C | C | R | S | S | R | | 65200 |
| 86 | 98 | Y | C | Y | S | L | Y | G | T | T | L | E | Q | Q | | 74500 |
| 7 | 19 | A | M | F | Q | D | P | Q | E | R | P | R | K | L | | 84200 |
| 128 | 140 | K | K | Q | R | F | H | N | I | R | G | R | W | T | | 110000 |
| 89 | 101 | S | L | Y | G | T | T | L | E | Q | Q | Y | N | K | | 155000 |
| 55 | 67 | R | D | L | C | I | V | Y | R | D | G | N | P | Y | | 162000 |
| 115 | 127 | K | P | L | C | P | E | E | K | Q | R | H | L | D | | 170000 |
| 103 | 115 | L | C | D | L | L | I | R | C | I | N | C | Q | K | | 180000 |
| 136 | 148 | R | G | R | W | T | G | R | C | M | S | C | C | R | | 182000 |
| 42 | 54 | Q | Q | L | L | R | R | E | V | Y | D | F | A | F | | 200000 |
| 77 | 89 | Y | S | K | I | S | E | Y | R | H | Y | C | Y | S | | 230000 |
| 145 | 157 | S | C | C | R | S | S | R | T | R | R | E | T | Q | | 245000 |
| 83 | 95 | Y | R | H | Y | C | Y | S | L | Y | G | T | T | L | | 265000 |
| 124 | 136 | R | H | L | D | K | K | Q | R | F | H | N | I | R | | 272000 |
| 87 | 99 | C | Y | S | L | Y | G | T | T | L | E | Q | Q | Y | | 305000 |
| 17 | 29 | R | K | L | P | Q | L | C | T | E | L | Q | T | T | | 390000 |
| 49 | 61 | V | Y | D | F | A | F | R | D | L | C | I | V | Y | | 415000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 107 | 119 | L | I | R | C | I | N | C | Q | K | P | L | C | P | 422000 |
| 35 | 47 | L | E | C | V | Y | C | K | Q | Q | L | L | R | R | 430000 |
| 53 | 65 | A | F | R | D | L | C | I | V | Y | R | D | G | N | 475000 |
| 39 | 51 | Y | C | K | Q | Q | L | L | R | R | E | V | Y | D | 480000 |
| 98 | 110 | Q | Y | N | K | P | L | C | D | L | L | I | R | C | 530000 |
| 125 | 137 | H | L | D | K | K | Q | R | F | H | N | I | R | G | 580000 |
| 30 | 42 | I | H | D | I | I | L | E | C | V | Y | C | K | Q | 612000 |
| 82 | 94 | E | Y | R | H | Y | C | Y | S | L | Y | G | T | T | 650000 |
| 143 | 155 | C | M | S | C | C | R | S | S | R | T | R | R | E | 770000 |
| 56 | 68 | D | L | C | I | V | Y | R | D | G | N | P | Y | A | 805000 |
| 1 | 13 | M | H | Q | K | R | T | A | M | F | Q | D | P | Q | 1000000 |
| 2 | 14 | H | Q | K | R | T | A | M | F | Q | D | P | Q | E | 1000000 |
| 3 | 15 | Q | K | R | T | A | M | F | Q | D | P | Q | E | R | 1000000 |
| 4 | 16 | K | R | T | A | M | F | Q | D | P | Q | E | R | P | 1000000 |
| 5 | 17 | R | T | A | M | F | Q | D | P | Q | E | R | P | R | 1000000 |
| 8 | 20 | M | F | Q | D | P | Q | E | R | P | R | K | L | P | 1000000 |
| 9 | 21 | F | Q | D | P | Q | E | R | P | R | K | L | P | Q | 1000000 |
| 10 | 22 | Q | D | P | Q | E | R | P | R | K | L | P | Q | L | 1000000 |
| 11 | 23 | D | P | Q | E | R | P | R | K | L | P | Q | L | C | 1000000 |
| 12 | 24 | P | Q | E | R | P | R | K | L | P | Q | L | C | T | 1000000 |
| 13 | 25 | Q | E | R | P | R | K | L | P | Q | L | C | T | E | 1000000 |
| 14 | 26 | E | R | P | R | K | L | P | Q | L | C | T | E | L | 1000000 |
| 15 | 27 | R | P | R | K | L | P | Q | L | C | T | E | L | Q | 1000000 |
| 16 | 28 | P | R | K | L | P | Q | L | C | T | E | L | Q | T | 1000000 |
| 18 | 30 | K | L | P | Q | L | C | T | E | L | Q | T | T | I | 1000000 |
| 19 | 31 | L | P | Q | L | C | T | E | L | Q | T | T | I | H | 1000000 |
| 20 | 32 | P | Q | L | C | T | E | L | Q | T | T | I | H | D | 1000000 |
| 21 | 33 | Q | L | C | T | E | L | Q | T | T | I | H | D | I | 1000000 |
| 22 | 34 | L | C | T | E | L | Q | T | T | I | H | D | I | I | 1000000 |
| 23 | 35 | C | T | E | L | Q | T | T | I | H | D | I | I | L | 1000000 |
| 25 | 37 | E | L | Q | T | T | I | H | D | I | I | L | E | C | 1000000 |
| 26 | 38 | L | Q | T | T | I | H | D | I | I | L | E | C | V | 1000000 |
| 27 | 39 | Q | T | T | I | H | D | I | I | L | E | C | V | Y | 1000000 |
| 29 | 41 | T | I | H | D | I | I | L | E | C | V | Y | C | K | 1000000 |
| 34 | 46 | I | L | E | C | V | Y | C | K | Q | Q | L | L | R | 1000000 |
| 38 | 50 | V | Y | C | K | Q | Q | L | L | R | R | E | V | Y | 1000000 |
| 40 | 52 | C | K | Q | Q | L | L | R | R | E | V | Y | D | F | 1000000 |
| 41 | 53 | K | Q | Q | L | L | R | R | E | V | Y | D | F | A | 1000000 |
| 44 | 56 | L | L | R | R | E | V | Y | D | F | A | F | R | D | 1000000 |
| 45 | 57 | L | R | R | E | V | Y | D | F | A | F | R | D | L | 1000000 |
| 46 | 58 | R | R | E | V | Y | D | F | A | F | R | D | L | C | 1000000 |
| 51 | 63 | D | F | A | F | R | D | L | C | I | V | Y | R | D | 1000000 |
| 54 | 66 | F | R | D | L | C | I | V | Y | R | D | G | N | P | 1000000 |
| 60 | 72 | V | Y | R | D | G | N | P | Y | A | V | C | D | K | 1000000 |
| 61 | 73 | Y | R | D | G | N | P | Y | A | V | C | D | K | C | 1000000 |
| 62 | 74 | R | D | G | N | P | Y | A | V | C | D | K | C | L | 1000000 |
| 63 | 75 | D | G | N | P | Y | A | V | C | D | K | C | L | K | 1000000 |
| 64 | 76 | G | N | P | Y | A | V | C | D | K | C | L | K | F | 1000000 |
| 66 | 78 | P | Y | A | V | C | D | K | C | L | K | F | Y | S | 1000000 |
| 68 | 80 | A | V | C | D | K | C | L | K | F | Y | S | K | I | 1000000 |
| 69 | 81 | V | C | D | K | C | L | K | F | Y | S | K | I | S | 1000000 |
| 70 | 82 | C | D | K | C | L | K | F | Y | S | K | I | S | E | 1000000 |
| 73 | 85 | C | L | K | F | Y | S | K | I | S | E | Y | R | H | 1000000 |
| 76 | 88 | F | Y | S | K | I | S | E | Y | R | H | Y | C | Y | 1000000 |
| 79 | 91 | K | I | S | E | Y | R | H | Y | C | Y | S | L | Y | 1000000 |
| 85 | 97 | H | Y | C | Y | S | L | Y | G | T | T | L | E | Q | 1000000 |
| 90 | 102 | L | Y | G | T | T | L | E | Q | Q | Y | N | K | P | 1000000 |
| 91 | 103 | Y | G | T | T | L | E | Q | Q | Y | N | K | P | L | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 92 | 104 | G | T | T | L | E | Q | Q | Y | N | K | P | L | C | 1000000 |
| 94 | 106 | T | L | E | Q | Q | Y | N | K | P | L | C | D | L | 1000000 |
| 95 | 107 | L | E | Q | Q | Y | N | K | P | L | C | D | L | L | 1000000 |
| 96 | 108 | E | Q | Q | Y | N | K | P | L | C | D | L | L | I | 1000000 |
| 99 | 111 | Y | N | K | P | L | C | D | L | L | I | R | C | I | 1000000 |
| 100 | 112 | N | K | P | L | C | D | L | L | I | R | C | I | N | 1000000 |
| 102 | 114 | P | L | C | D | L | L | I | R | C | I | N | C | Q | 1000000 |
| 108 | 120 | I | R | C | I | N | C | Q | K | P | L | C | P | E | 1000000 |
| 110 | 122 | C | I | N | C | Q | K | P | L | C | P | E | E | K | 1000000 |
| 111 | 123 | I | N | C | Q | K | P | L | C | P | E | E | K | Q | 1000000 |
| 112 | 124 | N | C | Q | K | P | L | C | P | E | E | K | Q | R | 1000000 |
| 113 | 125 | C | Q | K | P | L | C | P | E | E | K | Q | R | H | 1000000 |
| 114 | 126 | Q | K | P | L | C | P | E | E | K | Q | R | H | L | 1000000 |
| 116 | 128 | P | L | C | P | E | E | K | Q | R | H | L | D | K | 1000000 |
| 117 | 129 | L | C | P | E | E | K | Q | R | H | L | D | K | K | 1000000 |
| 118 | 130 | C | P | E | E | K | Q | R | H | L | D | K | K | Q | 1000000 |
| 119 | 131 | P | E | E | K | Q | R | H | L | D | K | K | Q | R | 1000000 |
| 120 | 132 | E | E | K | Q | R | H | L | D | K | K | Q | R | F | 1000000 |
| 121 | 133 | E | K | Q | R | H | L | D | K | K | Q | R | F | H | 1000000 |
| 122 | 134 | K | Q | R | H | L | D | K | K | Q | R | F | H | N | 1000000 |
| 123 | 135 | Q | R | H | L | D | K | K | Q | R | F | H | N | I | 1000000 |
| 126 | 138 | L | D | K | K | Q | R | F | H | N | I | R | G | R | 1000000 |
| 129 | 141 | K | Q | R | F | H | N | I | R | G | R | W | T | G | 1000000 |
| 131 | 143 | R | F | H | N | I | R | G | R | W | T | G | R | C | 1000000 |
| 132 | 144 | F | H | N | I | R | G | R | W | T | G | R | C | M | 1000000 |
| 134 | 146 | N | I | R | G | R | W | T | G | R | C | M | S | C | 1000000 |
| 135 | 147 | I | R | G | R | W | T | G | R | C | M | S | C | C | 1000000 |
| 138 | 150 | R | W | T | G | R | C | M | S | C | C | R | S | S | 1000000 |
| 141 | 153 | G | R | C | M | S | C | C | R | S | S | R | T | R | 1000000 |
| 144 | 156 | M | S | C | C | R | S | S | R | T | R | R | E | T | 1000000 |
| 146 | 158 | C | C | R | S | S | R | T | R | R | E | T | Q | L | 1000000 |

| | | | | | | | | | | | | | | | IC50 DRB1*0401 (nM) |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------------|
| 59 | 71 | I | V | Y | R | D | G | N | P | Y | A | V | C | D | 24 |
| 52 | 64 | F | A | F | R | D | L | C | I | V | Y | R | D | G | 35 |
| 75 | 87 | K | F | Y | S | K | I | S | E | Y | R | H | Y | C | 83 |
| 86 | 98 | Y | C | Y | S | L | Y | G | T | T | L | E | Q | Q | 120 |
| 31 | 43 | H | D | I | I | L | E | C | V | Y | C | K | Q | Q | 135 |
| 58 | 70 | C | I | V | Y | R | D | G | N | P | Y | A | V | C | 250 |
| 81 | 93 | S | E | Y | R | H | Y | C | Y | S | L | Y | G | T | 265 |
| 106 | 118 | L | L | I | R | C | I | N | C | Q | K | P | L | C | 290 |
| 43 | 55 | Q | L | L | R | R | E | V | Y | D | F | A | F | R | 360 |
| 142 | 154 | R | C | M | S | C | C | R | S | S | R | T | R | R | 370 |
| 133 | 145 | H | N | I | R | G | R | W | T | G | R | C | M | S | 425 |
| 109 | 121 | R | C | I | N | C | Q | K | P | L | C | P | E | E | 532 |
| 139 | 151 | W | T | G | R | C | M | S | C | C | R | S | S | R | 620 |
| 32 | 44 | D | I | I | L | E | C | V | Y | C | K | Q | Q | L | 660 |
| 28 | 40 | T | T | I | H | D | I | I | L | E | C | V | Y | C | 732 |
| 88 | 100 | Y | S | L | Y | G | T | T | L | E | Q | Q | Y | N | 862 |
| 105 | 117 | D | L | L | I | R | C | I | N | C | Q | K | P | L | 930 |
| 137 | 149 | G | R | W | T | G | R | C | M | S | C | C | R | S | 1550 |
| 37 | 49 | C | V | Y | C | K | Q | Q | L | L | R | R | E | V | 1600 |
| 78 | 90 | S | K | I | S | E | Y | R | H | Y | C | Y | S | L | 2200 |
| 74 | 86 | L | K | F | Y | S | K | I | S | E | Y | R | H | Y | 2500 |
| 50 | 62 | Y | D | F | A | F | R | D | L | C | I | V | Y | R | 2720 |
| 27 | 39 | Q | T | T | I | H | D | I | I | L | E | C | V | Y | 3400 |
| 48 | 60 | E | V | Y | D | F | A | F | R | D | L | C | I | V | 5300 |
| 82 | 94 | E | Y | R | H | Y | C | Y | S | L | Y | G | T | T | 6700 |
| 77 | 89 | Y | S | K | I | S | E | Y | R | H | Y | C | Y | S | 7300 |
| 130 | 142 | Q | R | F | H | N | I | R | G | R | W | T | G | R | 8620 |
| 65 | 77 | N | P | Y | A | V | C | D | K | C | L | K | F | Y | 10500 |
| 84 | 96 | R | H | Y | C | Y | S | L | Y | G | T | T | L | E | 10500 |
| 91 | 103 | Y | G | T | T | L | E | Q | Q | Y | N | K | P | L | 11000 |
| 103 | 115 | L | C | D | L | L | I | R | C | I | N | C | Q | K | 12000 |
| 24 | 36 | T | E | L | Q | T | T | I | H | D | I | I | L | E | 17500 |
| 104 | 116 | C | D | L | L | I | R | C | I | N | C | Q | K | P | 20000 |
| 57 | 69 | L | C | I | V | Y | R | D | G | N | P | Y | A | V | 20200 |
| 30 | 42 | I | H | D | I | I | L | E | C | V | Y | C | K | Q | 27000 |
| 33 | 45 | I | I | L | E | C | V | Y | C | K | Q | Q | L | L | 28000 |
| 47 | 59 | R | E | V | Y | D | F | A | F | R | D | L | C | I | 45200 |
| 72 | 84 | K | C | L | K | F | Y | S | K | I | S | E | Y | R | 48000 |
| 100 | 112 | N | K | P | L | C | D | L | L | I | R | C | I | N | 51500 |
| 20 | 32 | P | Q | L | C | T | E | L | Q | T | T | I | H | D | 54000 |
| 92 | 104 | G | T | T | L | E | Q | Q | Y | N | K | P | L | C | 60000 |
| 83 | 95 | Y | R | H | Y | C | Y | S | L | Y | G | T | T | L | 64500 |
| 6 | 18 | T | A | M | F | Q | D | P | Q | E | R | P | R | K | 67000 |
| 42 | 54 | Q | Q | L | L | R | R | E | V | Y | D | F | A | F | 68500 |
| 26 | 38 | L | Q | T | T | I | H | D | I | I | L | E | C | V | 75000 |
| 97 | 109 | Q | Q | Y | N | K | P | L | C | D | L | L | I | R | 79000 |
| 93 | 105 | T | T | L | E | Q | Q | Y | N | K | P | L | C | D | 81200 |
| 36 | 48 | E | C | V | Y | C | K | Q | Q | L | L | R | R | E | 82000 |
| 136 | 148 | R | G | R | W | T | G | R | C | M | S | C | C | R | 95200 |
| 87 | 99 | C | Y | S | L | Y | G | T | T | L | E | Q | Q | Y | 98000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 138 | 150 | R | W | T | G | R | C | M | S | C | C | R | S | S | 110000 |
| 80 | 92 | I | S | E | Y | R | H | Y | C | Y | S | L | Y | G | 115000 |
| 89 | 101 | S | L | Y | G | T | T | L | E | Q | Q | Y | N | K | 120000 |
| 127 | 139 | D | K | K | Q | R | F | H | N | I | R | G | R | W | 130000 |
| 4 | 16 | K | R | T | A | M | F | Q | D | P | Q | E | R | P | 175000 |
| 66 | 78 | P | Y | A | V | C | D | K | C | L | K | F | Y | S | 180000 |
| 135 | 147 | I | R | G | R | W | T | G | R | C | M | S | C | C | 225000 |
| 18 | 30 | K | L | P | Q | L | C | T | E | L | Q | T | T | I | 320000 |
| 16 | 28 | P | R | K | L | P | Q | L | C | T | E | L | Q | T | 390000 |
| 55 | 67 | R | D | L | C | I | V | Y | R | D | G | N | P | Y | 402000 |
| 85 | 97 | H | Y | C | Y | S | L | Y | G | T | T | L | E | Q | 472000 |
| 71 | 83 | D | K | C | L | K | F | Y | S | K | I | S | E | Y | 550000 |
| 90 | 102 | L | Y | G | T | T | L | E | Q | Q | Y | N | K | P | 615000 |
| 95 | 107 | L | E | Q | Q | Y | N | K | P | L | C | D | L | L | 760000 |
| 101 | 113 | K | P | L | C | D | L | L | I | R | C | I | N | C | 780000 |
| 108 | 120 | I | R | C | I | N | C | Q | K | P | L | C | P | E | 785000 |
| 54 | 66 | F | R | D | L | C | I | V | Y | R | D | G | N | P | 915000 |
| 1 | 13 | M | H | Q | K | R | T | A | M | F | Q | D | P | Q | 1000000 |
| 2 | 14 | H | Q | K | R | T | A | M | F | Q | D | P | Q | E | 1000000 |
| 3 | 15 | Q | K | R | T | A | M | F | Q | D | P | Q | E | R | 1000000 |
| 5 | 17 | R | T | A | M | F | Q | D | P | Q | E | R | P | R | 1000000 |
| 7 | 19 | A | M | F | Q | D | P | Q | E | R | P | R | K | L | 1000000 |
| 8 | 20 | M | F | Q | D | P | Q | E | R | P | R | K | L | P | 1000000 |
| 9 | 21 | F | Q | D | P | Q | E | R | P | R | K | L | P | Q | 1000000 |
| 10 | 22 | Q | D | P | Q | E | R | P | R | K | L | P | Q | L | 1000000 |
| 11 | 23 | D | P | Q | E | R | P | R | K | L | P | Q | L | C | 1000000 |
| 12 | 24 | P | Q | E | R | P | R | K | L | P | Q | L | C | T | 1000000 |
| 13 | 25 | Q | E | R | P | R | K | L | P | Q | L | C | T | E | 1000000 |
| 14 | 26 | E | R | P | R | K | L | P | Q | L | C | T | E | L | 1000000 |
| 15 | 27 | R | P | R | K | L | P | Q | L | C | T | E | L | Q | 1000000 |
| 17 | 29 | R | K | L | P | Q | L | C | T | E | L | Q | T | T | 1000000 |
| 19 | 31 | L | P | Q | L | C | T | E | L | Q | T | T | I | H | 1000000 |
| 21 | 33 | Q | L | C | T | E | L | Q | T | T | I | H | D | I | 1000000 |
| 22 | 34 | L | C | T | E | L | Q | T | T | I | H | D | I | I | 1000000 |
| 23 | 35 | C | T | E | L | Q | T | T | I | H | D | I | I | L | 1000000 |
| 25 | 37 | E | L | Q | T | T | I | H | D | I | I | L | E | C | 1000000 |
| 29 | 41 | T | I | H | D | I | I | L | E | C | V | Y | C | K | 1000000 |
| 34 | 46 | I | L | E | C | V | Y | C | K | Q | Q | L | L | R | 1000000 |
| 35 | 47 | L | E | C | V | Y | C | K | Q | Q | L | L | R | R | 1000000 |
| 38 | 50 | V | Y | C | K | Q | Q | L | L | R | R | E | V | Y | 1000000 |
| 39 | 51 | Y | C | K | Q | Q | L | L | R | R | E | V | Y | D | 1000000 |
| 40 | 52 | C | K | Q | Q | L | L | R | R | E | V | Y | D | F | 1000000 |
| 41 | 53 | K | Q | Q | L | L | R | R | E | V | Y | D | F | A | 1000000 |
| 44 | 56 | L | L | R | R | E | V | Y | D | F | A | F | R | D | 1000000 |
| 45 | 57 | L | R | R | E | V | Y | D | F | A | F | R | D | L | 1000000 |
| 46 | 58 | R | R | E | V | Y | D | F | A | F | R | D | L | C | 1000000 |
| 49 | 61 | V | Y | D | F | A | F | R | D | L | C | I | V | Y | 1000000 |
| 51 | 63 | D | F | A | F | R | D | L | C | I | V | Y | R | D | 1000000 |
| 53 | 65 | A | F | R | D | L | C | I | V | Y | R | D | G | N | 1000000 |
| 56 | 68 | D | L | C | I | V | Y | R | D | G | N | P | Y | A | 1000000 |
| 60 | 72 | V | Y | R | D | G | N | P | Y | A | V | C | D | K | 1000000 |

| | | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--|---------|
| 61 | 73 | Y | R | D | G | N | P | Y | A | V | C | D | K | C | | 1000000 |
| 62 | 74 | R | D | G | N | P | Y | A | V | C | D | K | C | L | | 1000000 |
| 63 | 75 | D | G | N | P | Y | A | V | C | D | K | C | L | K | | 1000000 |
| 64 | 76 | G | N | P | Y | A | V | C | D | K | C | L | K | F | | 1000000 |
| 67 | 79 | Y | A | V | C | D | K | C | L | K | F | Y | S | K | | 1000000 |
| 68 | 80 | A | V | C | D | K | C | L | K | F | Y | S | K | I | | 1000000 |
| 69 | 81 | V | C | D | K | C | L | K | F | Y | S | K | I | S | | 1000000 |
| 70 | 82 | C | D | K | C | L | K | F | Y | S | K | I | S | E | | 1000000 |
| 73 | 85 | C | L | K | F | Y | S | K | I | S | E | Y | R | H | | 1000000 |
| 76 | 88 | F | Y | S | K | I | S | E | Y | R | H | Y | C | Y | | 1000000 |
| 79 | 91 | K | I | S | E | Y | R | H | Y | C | Y | S | L | Y | | 1000000 |
| 94 | 106 | T | L | E | Q | Q | Y | N | K | P | L | C | D | L | | 1000000 |
| 96 | 108 | E | Q | Q | Y | N | K | P | L | C | D | L | L | I | | 1000000 |
| 98 | 110 | Q | Y | N | K | P | L | C | D | L | L | I | R | C | | 1000000 |
| 99 | 111 | Y | N | K | P | L | C | D | L | L | I | R | C | I | | 1000000 |
| 102 | 114 | P | L | C | D | L | L | I | R | C | I | N | C | Q | | 1000000 |
| 107 | 119 | L | I | R | C | I | N | C | Q | K | P | L | C | P | | 1000000 |
| 110 | 122 | C | I | N | C | Q | K | P | L | C | P | E | E | K | | 1000000 |
| 111 | 123 | I | N | C | Q | K | P | L | C | P | E | E | K | Q | | 1000000 |
| 112 | 124 | N | C | Q | K | P | L | C | P | E | E | K | Q | R | | 1000000 |
| 113 | 125 | C | Q | K | P | L | C | P | E | E | K | Q | R | H | | 1000000 |
| 114 | 126 | Q | K | P | L | C | P | E | E | K | Q | R | H | L | | 1000000 |
| 115 | 127 | K | P | L | C | P | E | E | K | Q | R | H | L | D | | 1000000 |
| 116 | 128 | P | L | C | P | E | E | K | Q | R | H | L | D | K | | 1000000 |
| 117 | 129 | L | C | P | E | E | K | Q | R | H | L | D | K | K | | 1000000 |
| 118 | 130 | C | P | E | E | K | Q | R | H | L | D | K | K | Q | | 1000000 |
| 119 | 131 | P | E | E | K | Q | R | H | L | D | K | K | Q | R | | 1000000 |
| 120 | 132 | E | E | K | Q | R | H | L | D | K | K | Q | R | F | | 1000000 |
| 121 | 133 | E | K | Q | R | H | L | D | K | K | Q | R | F | H | | 1000000 |
| 122 | 134 | K | Q | R | H | L | D | K | K | Q | R | F | H | N | | 1000000 |
| 123 | 135 | Q | R | H | L | D | K | K | Q | R | F | H | N | I | | 1000000 |
| 124 | 136 | R | H | L | D | K | K | Q | R | F | H | N | I | R | | 1000000 |
| 125 | 137 | H | L | D | K | K | Q | R | F | H | N | I | R | G | | 1000000 |
| 126 | 138 | L | D | K | K | Q | R | F | H | N | I | R | G | R | | 1000000 |
| 128 | 140 | K | K | Q | R | F | H | N | I | R | G | R | W | T | | 1000000 |
| 129 | 141 | K | Q | R | F | H | N | I | R | G | R | W | T | G | | 1000000 |
| 131 | 143 | R | F | H | N | I | R | G | R | W | T | G | R | C | | 1000000 |
| 132 | 144 | F | H | N | I | R | G | R | W | T | G | R | C | M | | 1000000 |
| 134 | 146 | N | I | R | G | R | W | T | G | R | C | M | S | C | | 1000000 |
| 140 | 152 | T | G | R | C | M | S | C | C | R | S | S | R | T | | 1000000 |
| 141 | 153 | G | R | C | M | S | C | C | R | S | S | R | T | R | | 1000000 |
| 143 | 155 | C | M | S | C | C | R | S | S | R | T | R | R | E | | 1000000 |
| 144 | 156 | M | S | C | C | R | S | S | R | T | R | R | E | T | | 1000000 |
| 145 | 157 | S | C | C | R | S | S | R | T | R | R | E | T | Q | | 1000000 |
| 146 | 158 | C | C | R | S | S | R | T | R | R | E | T | Q | L | | 1000000 |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | IC50 DRB1*0101(nM) |
|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------------------|
| 81 | 93 | D | L | L | M | G | T | L | G | I | V | C | P | I | 45.2 |
| 80 | 92 | E | D | L | L | M | G | T | L | G | I | V | C | P | 230 |
| 52 | 64 | Y | N | I | V | T | F | C | C | K | C | D | S | T | 300 |
| 55 | 67 | V | T | F | C | C | K | C | D | S | T | L | R | L | 1120 |
| 10 | 22 | E | Y | M | L | D | L | Q | P | E | T | T | D | L | 1600 |
| 77 | 89 | R | T | L | E | D | L | L | M | G | T | L | G | I | 1800 |
| 74 | 86 | V | D | I | R | T | L | E | D | L | L | M | G | T | 2200 |
| 21 | 33 | D | L | Y | C | Y | E | Q | L | N | D | S | S | E | 4550 |
| 63 | 75 | S | T | L | R | L | C | V | Q | S | T | H | V | D | 5600 |
| 50 | 62 | A | H | Y | N | I | V | T | F | C | C | K | C | D | 8020 |
| 67 | 79 | L | C | V | Q | S | T | H | V | D | I | R | T | L | 8950 |
| 36 | 48 | D | E | I | D | G | P | A | G | Q | A | E | P | D | 9420 |
| 65 | 77 | L | R | L | C | V | Q | S | T | H | V | D | I | R | 11000 |
| 20 | 32 | T | D | L | Y | C | Y | E | Q | L | N | D | S | S | 13000 |
| 79 | 91 | L | E | D | L | L | M | G | T | L | G | I | V | C | 18500 |
| 9 | 21 | H | E | Y | M | L | D | L | Q | P | E | T | T | D | 25000 |
| 85 | 97 | G | T | L | G | I | V | C | P | I | C | S | Q | K | 28200 |
| 23 | 35 | Y | C | Y | E | Q | L | N | D | S | S | E | E | E | 28500 |
| 46 | 58 | E | P | D | R | A | H | Y | N | I | V | T | F | C | 33500 |
| 35 | 47 | E | D | E | I | D | G | P | A | G | Q | A | E | P | 47500 |
| 83 | 95 | L | M | G | T | L | G | I | V | C | P | I | C | S | 51000 |
| 82 | 94 | L | L | M | G | T | L | G | I | V | C | P | I | C | 56500 |
| 53 | 65 | N | I | V | T | F | C | C | K | C | D | S | T | L | 60000 |
| 17 | 29 | P | E | T | T | D | L | Y | C | Y | E | Q | L | N | 64500 |
| 62 | 74 | D | S | T | L | R | L | C | V | Q | S | T | H | V | 67500 |
| 57 | 69 | F | C | C | K | C | D | S | T | L | R | L | C | V | 97500 |
| 13 | 25 | L | D | L | Q | P | E | T | T | D | L | Y | C | Y | 140000 |
| 51 | 63 | H | Y | N | I | V | T | F | C | C | K | C | D | S | 315000 |
| 59 | 71 | C | K | C | D | S | T | L | R | L | C | V | Q | S | 405000 |
| 84 | 96 | M | G | T | L | G | I | V | C | P | I | C | S | Q | 410000 |
| 6 | 18 | P | T | L | H | E | Y | M | L | D | L | Q | P | E | 435000 |
| 58 | 70 | C | C | K | C | D | S | T | L | R | L | C | V | Q | 572000 |
| 72 | 84 | T | H | V | D | I | R | T | L | E | D | L | L | M | 670000 |
| 69 | 81 | V | Q | S | T | H | V | D | I | R | T | L | E | D | 720000 |
| 60 | 72 | K | C | D | S | T | L | R | L | C | V | Q | S | T | 815000 |
| 61 | 73 | C | D | S | T | L | R | L | C | V | Q | S | T | H | 850000 |
| 1 | 13 | M | H | G | D | T | P | T | L | H | E | Y | M | L | 1000000 |
| 2 | 14 | H | G | D | T | P | T | L | H | E | Y | M | L | D | 1000000 |
| 3 | 15 | G | D | T | P | T | L | H | E | Y | M | L | D | L | 1000000 |
| 4 | 16 | D | T | P | T | L | H | E | Y | M | L | D | L | Q | 1000000 |
| 5 | 17 | T | P | T | L | H | E | Y | M | L | D | L | Q | P | 1000000 |
| 7 | 19 | T | L | H | E | Y | M | L | D | L | Q | P | E | T | 1000000 |
| 8 | 20 | L | H | E | Y | M | L | D | L | Q | P | E | T | T | 1000000 |
| 11 | 23 | Y | M | L | D | L | Q | P | E | T | T | D | L | Y | 1000000 |
| 12 | 24 | M | L | D | L | Q | P | E | T | T | D | L | Y | C | 1000000 |
| 14 | 26 | D | L | Q | P | E | T | T | D | L | Y | C | Y | E | 1000000 |
| 15 | 27 | L | Q | P | E | T | T | D | L | Y | C | Y | E | Q | 1000000 |
| 16 | 28 | Q | P | E | T | T | D | L | Y | C | Y | E | Q | L | 1000000 |
| 18 | 30 | E | T | T | D | L | Y | C | Y | E | Q | L | N | D | 1000000 |
| 19 | 31 | T | T | D | L | Y | C | Y | E | Q | L | N | D | S | 1000000 |

| | | | | | | | | | | | | | | | |
|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 22 | 34 | L | Y | C | Y | E | Q | L | N | D | S | S | E | E | 1000000 |
| 24 | 36 | C | Y | E | Q | L | N | D | S | S | E | E | E | D | 1000000 |
| 25 | 37 | Y | E | Q | L | N | D | S | S | E | E | E | D | E | 1000000 |
| 26 | 38 | E | Q | L | N | D | S | S | E | E | E | D | E | I | 1000000 |
| 27 | 39 | Q | L | N | D | S | S | E | E | E | D | E | I | D | 1000000 |
| 28 | 40 | L | N | D | S | S | E | E | E | D | E | I | D | G | 1000000 |
| 29 | 41 | N | D | S | S | E | E | E | D | E | I | D | G | P | 1000000 |
| 30 | 42 | D | S | S | E | E | E | D | E | I | D | G | P | A | 1000000 |
| 31 | 43 | S | S | E | E | E | D | E | I | D | G | P | A | G | 1000000 |
| 32 | 44 | S | E | E | E | D | E | I | D | G | P | A | G | Q | 1000000 |
| 33 | 45 | E | E | E | D | E | I | D | G | P | A | G | Q | A | 1000000 |
| 34 | 46 | E | E | D | E | I | D | G | P | A | G | Q | A | E | 1000000 |
| 37 | 49 | E | I | D | G | P | A | G | Q | A | E | P | D | R | 1000000 |
| 38 | 50 | I | D | G | P | A | G | Q | A | E | P | D | R | A | 1000000 |
| 39 | 51 | D | G | P | A | G | Q | A | E | P | D | R | A | H | 1000000 |
| 40 | 52 | G | P | A | G | Q | A | E | P | D | R | A | H | Y | 1000000 |
| 41 | 53 | P | A | G | Q | A | E | P | D | R | A | H | Y | N | 1000000 |
| 42 | 54 | A | G | Q | A | E | P | D | R | A | H | Y | N | I | 1000000 |
| 43 | 55 | G | Q | A | E | P | D | R | A | H | Y | N | I | V | 1000000 |
| 44 | 56 | Q | A | E | P | D | R | A | H | Y | N | I | V | T | 1000000 |
| 45 | 57 | A | E | P | D | R | A | H | Y | N | I | V | T | F | 1000000 |
| 47 | 59 | P | D | R | A | H | Y | N | I | V | T | F | C | C | 1000000 |
| 48 | 60 | D | R | A | H | Y | N | I | V | T | F | C | C | K | 1000000 |
| 49 | 61 | R | A | H | Y | N | I | V | T | F | C | C | K | C | 1000000 |
| 54 | 66 | I | V | T | F | C | C | K | C | D | S | T | L | R | 1000000 |
| 56 | 68 | T | F | C | C | K | C | D | S | T | L | R | L | C | 1000000 |
| 64 | 76 | T | L | R | L | C | V | Q | S | T | H | V | D | I | 1000000 |
| 66 | 78 | R | L | C | V | Q | S | T | H | V | D | I | R | T | 1000000 |
| 68 | 80 | C | V | Q | S | T | H | V | D | I | R | T | L | E | 1000000 |
| 70 | 82 | Q | S | T | H | V | D | I | R | T | L | E | D | L | 1000000 |
| 71 | 83 | S | T | H | V | D | I | R | T | L | E | D | L | L | 1000000 |
| 73 | 85 | H | V | D | I | R | T | L | E | D | L | L | M | G | 1000000 |
| 75 | 87 | D | I | R | T | L | E | D | L | L | M | G | T | L | 1000000 |
| 76 | 88 | I | R | T | L | E | D | L | L | M | G | T | L | G | 1000000 |
| 78 | 90 | T | L | E | D | L | L | M | G | T | L | G | I | V | 1000000 |
| 86 | 98 | T | L | G | I | V | C | P | I | C | S | Q | K | P | 1000000 |

| | | | | | | | | | | | | | | | | IC50 DRB1*0401 (nM) |
|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|--|---------------------|
| 21 | 33 | D | L | Y | C | Y | E | Q | L | N | D | S | S | E | | 105 |
| 9 | 21 | H | E | Y | M | L | D | L | Q | P | E | T | T | D | | 210 |
| 84 | 96 | M | G | T | L | G | I | V | C | P | I | C | S | Q | | 230 |
| 63 | 75 | S | T | L | R | L | C | V | Q | S | T | H | V | D | | 452 |
| 81 | 93 | D | L | L | M | G | T | L | G | I | V | C | P | I | | 710 |
| 85 | 97 | G | T | L | G | I | V | C | P | I | C | S | Q | K | | 920 |
| 53 | 65 | N | I | V | T | F | C | C | K | C | D | S | T | L | | 1700 |
| 80 | 92 | E | D | L | L | M | G | T | L | G | I | V | C | P | | 1800 |
| 50 | 62 | A | H | Y | N | I | V | T | F | C | C | K | C | D | | 2600 |
| 74 | 86 | V | D | I | R | T | L | E | D | L | L | M | G | T | | 2650 |
| 67 | 79 | L | C | V | Q | S | T | H | V | D | I | R | T | L | | 3700 |
| 17 | 29 | P | E | T | T | D | L | Y | C | Y | E | Q | L | N | | 4750 |
| 82 | 94 | L | L | M | G | T | L | G | I | V | C | P | I | C | | 5120 |
| 65 | 77 | L | R | L | C | V | Q | S | T | H | V | D | I | R | | 5600 |
| 20 | 32 | T | D | L | Y | C | Y | E | Q | L | N | D | S | S | | 6720 |
| 48 | 60 | D | R | A | H | Y | N | I | V | T | F | C | C | K | | 7120 |
| 13 | 25 | L | D | L | Q | P | E | T | T | D | L | Y | C | Y | | 7300 |
| 10 | 22 | E | Y | M | L | D | L | Q | P | E | T | T | D | L | | 9320 |
| 6 | 18 | P | T | L | H | E | Y | M | L | D | L | Q | P | E | | 9700 |
| 54 | 66 | I | V | T | F | C | C | K | C | D | S | T | L | R | | 11500 |
| 52 | 64 | Y | N | I | V | T | F | C | C | K | C | D | S | T | | 16500 |
| 51 | 63 | H | Y | N | I | V | T | F | C | C | K | C | D | S | | 30000 |
| 83 | 95 | L | M | G | T | L | G | I | V | C | P | I | C | S | | 53000 |
| 57 | 69 | F | C | C | K | C | D | S | T | L | R | L | C | V | | 60000 |
| 55 | 67 | V | T | F | C | C | K | C | D | S | T | L | R | L | | 62500 |
| 75 | 87 | D | I | R | T | L | E | D | L | L | M | G | T | L | | 68000 |
| 49 | 61 | R | A | H | Y | N | I | V | T | F | C | C | K | C | | 76200 |
| 5 | 17 | T | P | T | L | H | E | Y | M | L | D | L | Q | P | | 96500 |
| 79 | 91 | L | E | D | L | L | M | G | T | L | G | I | V | C | | 110000 |
| 46 | 58 | E | P | D | R | A | H | Y | N | I | V | T | F | C | | 122000 |
| 64 | 76 | T | L | R | L | C | V | Q | S | T | H | V | D | I | | 132000 |
| 76 | 88 | I | R | T | L | E | D | L | L | M | G | T | L | G | | 145000 |
| 86 | 98 | T | L | G | I | V | C | P | I | C | S | Q | K | P | | 202000 |
| 77 | 89 | R | T | L | E | D | L | L | M | G | T | L | G | I | | 260000 |
| 18 | 30 | E | T | T | D | L | Y | C | Y | E | Q | L | N | D | | 535000 |
| 62 | 74 | D | S | T | L | R | L | C | V | Q | S | T | H | V | | 580000 |
| 26 | 38 | E | Q | L | N | D | S | S | E | E | E | D | E | I | | 590000 |
| 60 | 72 | K | C | D | S | T | L | R | L | C | V | Q | S | T | | 695000 |
| 71 | 83 | S | T | H | V | D | I | R | T | L | E | D | L | L | | 870000 |
| 24 | 36 | C | Y | E | Q | L | N | D | S | S | E | E | E | D | | 952000 |
| 1 | 13 | M | H | G | D | T | P | T | L | H | E | Y | M | L | | 1000000 |
| 2 | 14 | H | G | D | T | P | T | L | H | E | Y | M | L | D | | 1000000 |
| 3 | 15 | G | D | T | P | T | L | H | E | Y | M | L | D | L | | 1000000 |
| 4 | 16 | D | T | P | T | L | H | E | Y | M | L | D | L | Q | | 1000000 |
| 7 | 19 | T | L | H | E | Y | M | L | D | L | Q | P | E | T | | 1000000 |
| 8 | 20 | L | H | E | Y | M | L | D | L | Q | P | E | T | T | | 1000000 |
| 11 | 23 | Y | M | L | D | L | Q | P | E | T | T | D | L | Y | | 1000000 |
| 12 | 24 | M | L | D | L | Q | P | E | T | T | D | L | Y | C | | 1000000 |
| 14 | 26 | D | L | Q | P | E | T | T | D | L | Y | C | Y | E | | 1000000 |
| 15 | 27 | L | Q | P | E | T | T | D | L | Y | C | Y | E | Q | | 1000000 |

| | | | | | | | | | | | | | | | |
|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 16 | 28 | Q | P | E | T | T | D | L | Y | C | Y | E | Q | L | 1000000 |
| 19 | 31 | T | T | D | L | Y | C | Y | E | Q | L | N | D | S | 1000000 |
| 22 | 34 | L | Y | C | Y | E | Q | L | N | D | S | S | E | E | 1000000 |
| 23 | 35 | Y | C | Y | E | Q | L | N | D | S | S | E | E | E | 1000000 |
| 25 | 37 | Y | E | Q | L | N | D | S | S | E | E | E | D | E | 1000000 |
| 27 | 39 | Q | L | N | D | S | S | E | E | E | D | E | I | D | 1000000 |
| 28 | 40 | L | N | D | S | S | E | E | E | D | E | I | D | G | 1000000 |
| 29 | 41 | N | D | S | S | E | E | E | D | E | I | D | G | P | 1000000 |
| 30 | 42 | D | S | S | E | E | E | D | E | I | D | G | P | A | 1000000 |
| 31 | 43 | S | S | E | E | E | D | E | I | D | G | P | A | G | 1000000 |
| 32 | 44 | S | E | E | E | D | E | I | D | G | P | A | G | Q | 1000000 |
| 33 | 45 | E | E | E | D | E | I | D | G | P | A | G | Q | A | 1000000 |
| 34 | 46 | E | E | D | E | I | D | G | P | A | G | Q | A | E | 1000000 |
| 35 | 47 | E | D | E | I | D | G | P | A | G | Q | A | E | P | 1000000 |
| 36 | 48 | D | E | I | D | G | P | A | G | Q | A | E | P | D | 1000000 |
| 37 | 49 | E | I | D | G | P | A | G | Q | A | E | P | D | R | 1000000 |
| 38 | 50 | I | D | G | P | A | G | Q | A | E | P | D | R | A | 1000000 |
| 39 | 51 | D | G | P | A | G | Q | A | E | P | D | R | A | H | 1000000 |
| 40 | 52 | G | P | A | G | Q | A | E | P | D | R | A | H | Y | 1000000 |
| 41 | 53 | P | A | G | Q | A | E | P | D | R | A | H | Y | N | 1000000 |
| 42 | 54 | A | G | Q | A | E | P | D | R | A | H | Y | N | I | 1000000 |
| 43 | 55 | G | Q | A | E | P | D | R | A | H | Y | N | I | V | 1000000 |
| 44 | 56 | Q | A | E | P | D | R | A | H | Y | N | I | V | T | 1000000 |
| 45 | 57 | A | E | P | D | R | A | H | Y | N | I | V | T | F | 1000000 |
| 47 | 59 | P | D | R | A | H | Y | N | I | V | T | F | C | C | 1000000 |
| 56 | 68 | T | F | C | C | K | C | D | S | T | L | R | L | C | 1000000 |
| 58 | 70 | C | C | K | C | D | S | T | L | R | L | C | V | Q | 1000000 |
| 59 | 71 | C | K | C | D | S | T | L | R | L | C | V | Q | S | 1000000 |
| 61 | 73 | C | D | S | T | L | R | L | C | V | Q | S | T | H | 1000000 |
| 66 | 78 | R | L | C | V | Q | S | T | H | V | D | I | R | T | 1000000 |
| 68 | 80 | C | V | Q | S | T | H | V | D | I | R | T | L | E | 1000000 |
| 69 | 81 | V | Q | S | T | H | V | D | I | R | T | L | E | D | 1000000 |
| 70 | 82 | Q | S | T | H | V | D | I | R | T | L | E | D | L | 1000000 |
| 72 | 84 | T | H | V | D | I | R | T | L | E | D | L | L | M | 1000000 |
| 73 | 85 | H | V | D | I | R | T | L | E | D | L | L | M | G | 1000000 |
| 78 | 90 | T | L | E | D | L | L | M | G | T | L | G | I | V | 1000000 |

| | | | | | | | | | | | | | | | IC50 DRB1*0101 (nM) |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------------|
| 58 | 70 | N | I | Y | Y | H | A | G | T | S | R | L | L | A | 1.5 |
| 3 | 15 | V | T | F | I | Y | I | L | V | I | T | C | Y | E | 1.85 |
| 398 | 410 | L | Q | F | I | F | Q | L | C | K | I | T | L | T | 2.85 |
| 414 | 426 | M | T | Y | I | H | S | M | N | S | T | I | L | E | 3.25 |
| 442 | 454 | D | T | Y | R | F | V | T | Q | A | I | A | C | Q | 4.42 |
| 300 | 312 | D | L | Y | I | K | G | S | G | S | T | A | N | L | 5.05 |
| 315 | 327 | S | N | Y | F | P | T | P | S | G | S | M | V | T | 6.4 |
| 59 | 71 | I | Y | Y | H | A | G | T | S | R | L | L | A | V | 7.25 |
| 159 | 171 | S | A | Y | A | A | N | A | G | V | D | N | R | E | 22.5 |
| 21 | 33 | H | H | I | F | F | Q | M | S | L | W | L | P | S | 28.5 |
| 124 | 136 | L | V | W | A | C | V | G | V | E | V | G | R | G | 28.5 |
| 234 | 246 | M | D | F | T | T | L | Q | A | N | K | S | E | V | 33 |
| 5 | 17 | F | I | Y | I | L | V | I | T | C | Y | E | N | D | 36 |
| 468 | 480 | K | K | Y | T | F | W | E | V | N | L | K | E | K | 36 |
| 273 | 285 | F | F | Y | L | R | R | E | Q | M | F | V | R | H | 54 |
| 280 | 292 | Q | M | F | V | R | H | L | F | N | R | A | G | T | 57 |
| 67 | 79 | R | L | L | A | V | G | H | P | Y | F | P | I | K | 65.5 |
| 1 | 13 | M | Q | V | T | F | I | Y | I | L | V | I | T | C | 67.5 |
| 281 | 293 | M | F | V | R | H | L | F | N | R | A | G | T | V | 84 |
| 94 | 106 | L | Q | Y | R | V | F | R | I | H | L | P | D | P | 86 |
| 175 | 187 | M | D | Y | K | Q | T | Q | L | C | L | I | G | C | 89.5 |
| 22 | 34 | H | I | F | F | Q | M | S | L | W | L | P | S | E | 90.2 |
| 45 | 57 | S | K | V | V | S | T | D | E | Y | V | A | R | T | 92 |
| 366 | 378 | T | N | M | S | L | C | A | A | I | S | T | S | E | 93 |
| 114 | 126 | T | S | F | Y | N | P | D | T | Q | R | L | V | W | 96.5 |
| 212 | 224 | P | P | L | E | L | I | N | T | V | I | Q | D | G | 110 |
| 337 | 349 | Y | W | L | Q | R | A | Q | G | H | N | N | G | I | 115 |
| 504 | 516 | P | K | F | T | L | G | K | R | K | A | T | P | T | 115 |
| 123 | 135 | R | L | V | W | A | C | V | G | V | E | V | G | R | 120 |
| 52 | 64 | E | Y | V | A | R | T | N | I | Y | Y | H | A | G | 122 |
| 335 | 347 | K | P | Y | W | L | Q | R | A | Q | G | H | N | N | 130 |
| 84 | 96 | N | K | I | L | V | P | K | V | S | G | L | Q | Y | 150 |
| 492 | 504 | R | K | F | L | L | Q | A | G | L | K | A | K | P | 150 |
| 76 | 88 | F | P | I | K | K | P | N | N | N | K | I | L | V | 152 |
| 405 | 417 | C | K | I | T | L | T | A | D | V | M | T | Y | I | 152 |
| 6 | 18 | I | Y | I | L | V | I | T | C | Y | E | N | D | V | 160 |
| 85 | 97 | K | I | L | V | P | K | V | S | G | L | Q | Y | R | 165 |
| 150 | 162 | N | K | L | D | D | T | E | N | A | S | A | Y | A | 190 |
| 285 | 297 | H | L | F | N | R | A | G | T | V | G | E | N | V | 195 |
| 23 | 35 | I | F | F | Q | M | S | L | W | L | P | S | E | A | 200 |
| 115 | 127 | S | F | Y | N | P | D | T | Q | R | L | V | W | A | 205 |
| 412 | 424 | D | V | M | T | Y | I | H | S | M | N | S | T | I | 240 |
| 51 | 63 | D | E | Y | V | A | R | T | N | I | Y | Y | H | A | 260 |
| 129 | 141 | V | G | V | E | V | G | R | G | Q | P | L | G | V | 265 |
| 12 | 24 | T | C | Y | E | N | D | V | N | V | H | H | I | F | 432 |
| 9 | 21 | L | V | I | T | C | Y | E | N | D | V | N | V | H | 505 |
| 248 | 260 | L | D | I | C | T | S | I | C | K | Y | P | D | Y | 540 |
| 394 | 406 | E | E | Y | D | L | Q | F | I | F | Q | L | C | K | 540 |
| 271 | 283 | S | L | F | F | Y | L | R | R | E | Q | M | F | V | 550 |
| 445 | 457 | R | F | V | T | Q | A | I | A | C | Q | K | H | T | 570 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|-------|
| 270 | 282 | D | S | L | F | F | Y | L | R | R | E | Q | M | F | 582 |
| 299 | 311 | D | D | L | Y | I | K | G | S | G | S | T | A | N | 630 |
| 36 | 48 | T | V | Y | L | P | P | V | P | V | S | K | V | V | 680 |
| 347 | 359 | N | G | I | C | W | G | N | Q | L | F | V | T | V | 700 |
| 182 | 194 | L | C | L | I | G | C | K | P | P | I | G | E | H | 835 |
| 411 | 423 | A | D | V | M | T | Y | I | H | S | M | N | S | T | 845 |
| 27 | 39 | M | S | L | W | L | P | S | E | A | T | V | Y | L | 860 |
| 430 | 442 | F | G | L | Q | P | P | P | G | G | T | L | E | D | 860 |
| 173 | 185 | I | S | M | D | Y | K | Q | T | Q | L | C | L | I | 912 |
| 224 | 236 | G | D | M | V | H | T | G | F | G | A | M | D | F | 1720 |
| 89 | 101 | P | K | V | S | G | L | Q | Y | R | V | F | R | I | 1900 |
| 258 | 270 | P | D | Y | L | K | M | V | S | E | P | Y | G | D | 1950 |
| 423 | 435 | T | I | L | E | D | W | N | F | G | L | Q | P | P | 2250 |
| 237 | 249 | T | T | L | Q | A | N | K | S | E | V | P | L | D | 2500 |
| 336 | 348 | P | Y | W | L | Q | R | A | Q | G | H | N | N | G | 2620 |
| 225 | 237 | D | M | V | H | T | G | F | G | A | M | D | F | T | 2650 |
| 399 | 411 | Q | F | I | F | Q | L | C | K | I | T | L | T | A | 2800 |
| 353 | 365 | N | Q | L | F | V | T | V | V | D | T | T | R | S | 2950 |
| 331 | 343 | Q | I | F | N | K | P | Y | W | L | Q | R | A | Q | 3050 |
| 193 | 205 | E | H | W | G | K | G | S | P | C | T | N | V | A | 3250 |
| 310 | 322 | A | N | L | A | S | S | N | Y | F | P | T | P | S | 3300 |
| 180 | 192 | T | Q | L | C | L | I | G | C | K | P | P | I | G | 3600 |
| 444 | 456 | Y | R | F | V | T | Q | A | I | A | C | Q | K | H | 3700 |
| 330 | 342 | A | Q | I | F | N | K | P | Y | W | L | Q | R | A | 3720 |
| 357 | 369 | V | T | V | V | D | T | T | R | S | T | N | M | S | 3750 |
| 18 | 30 | V | N | V | H | H | I | F | F | Q | M | S | L | W | 3800 |
| 246 | 258 | V | P | L | D | I | C | T | S | I | C | K | Y | P | 4150 |
| 323 | 335 | G | S | M | V | T | S | D | A | Q | I | F | N | K | 4200 |
| 426 | 438 | E | D | W | N | F | G | L | Q | P | P | P | G | G | 4350 |
| 219 | 231 | T | V | I | Q | D | G | D | M | V | H | T | G | F | 4550 |
| 202 | 214 | T | N | V | A | V | N | P | G | D | C | P | P | L | 4750 |
| 141 | 153 | V | G | I | S | G | H | P | L | L | N | K | L | D | 5020 |
| 379 | 391 | T | T | Y | K | N | T | N | F | K | E | Y | L | R | 5050 |
| 122 | 134 | Q | R | L | V | W | A | C | V | G | V | E | V | G | 5320 |
| 8 | 20 | I | L | V | I | T | C | Y | E | N | D | V | N | V | 5400 |
| 25 | 37 | F | Q | M | S | L | W | L | P | S | E | A | T | V | 5450 |
| 88 | 100 | V | P | K | V | S | G | L | Q | Y | R | V | F | R | 5500 |
| 231 | 243 | F | G | A | M | D | F | T | T | L | Q | A | N | K | 5750 |
| 396 | 408 | Y | D | L | Q | F | I | F | Q | L | C | K | I | T | 5900 |
| 329 | 341 | D | A | Q | I | F | N | K | P | Y | W | L | Q | R | 5950 |
| 252 | 264 | T | S | I | C | K | Y | P | D | Y | L | K | M | V | 6250 |
| 16 | 28 | N | D | V | N | V | H | H | I | F | F | Q | M | S | 6600 |
| 131 | 143 | V | E | V | G | R | G | Q | P | L | G | V | G | I | 7220 |
| 261 | 273 | L | K | M | V | S | E | P | Y | G | D | S | L | F | 7220 |
| 364 | 376 | R | S | T | N | M | S | L | C | A | A | I | S | T | 7600 |
| 92 | 104 | S | G | L | Q | Y | R | V | F | R | I | H | L | P | 7920 |
| 470 | 482 | Y | T | F | W | E | V | N | L | K | E | K | F | S | 8150 |
| 165 | 177 | A | G | V | D | N | R | E | C | I | S | M | D | Y | 8320 |
| 483 | 495 | A | D | L | D | Q | F | P | L | G | R | K | F | L | 8600 |
| 138 | 150 | P | L | G | V | G | I | S | G | H | P | L | L | N | 9950 |
| 365 | 377 | S | T | N | M | S | L | C | A | A | I | S | T | S | 11000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|-------|
| 97 | 109 | R | V | F | R | I | H | L | P | D | P | N | K | F | 12500 |
| 349 | 361 | I | C | W | G | N | Q | L | F | V | T | V | V | D | 12500 |
| 303 | 315 | I | K | G | S | G | S | T | A | N | L | A | S | S | 13000 |
| 384 | 396 | T | N | F | K | E | Y | L | R | H | G | E | E | Y | 13000 |
| 479 | 491 | E | K | F | S | A | D | L | D | Q | F | P | L | G | 13000 |
| 493 | 505 | K | F | L | L | Q | A | G | L | K | A | K | P | K | 13000 |
| 137 | 149 | Q | P | L | G | V | G | I | S | G | H | P | L | L | 14500 |
| 400 | 412 | F | I | F | Q | L | C | K | I | T | L | T | A | D | 15200 |
| 465 | 477 | D | P | L | K | K | Y | T | F | W | E | V | N | L | 15200 |
| 441 | 453 | E | D | T | Y | R | F | V | T | Q | A | I | A | C | 16000 |
| 73 | 85 | H | P | Y | F | P | I | K | K | P | N | N | N | K | 17000 |
| 127 | 139 | A | C | V | G | V | E | V | G | R | G | Q | P | L | 17000 |
| 155 | 167 | T | E | N | A | S | A | Y | A | A | N | A | G | V | 17200 |
| 388 | 400 | E | Y | L | R | H | G | E | E | Y | D | L | Q | F | 19200 |
| 34 | 46 | E | A | T | V | Y | L | P | P | V | P | V | S | K | 19500 |
| 387 | 399 | K | E | Y | L | R | H | G | E | E | Y | D | L | Q | 21000 |
| 438 | 450 | G | T | L | E | D | T | Y | R | F | V | T | Q | A | 21000 |
| 156 | 168 | E | N | A | S | A | Y | A | A | N | A | G | V | D | 21500 |
| 171 | 183 | E | C | I | S | M | D | Y | K | Q | T | Q | L | C | 21500 |
| 153 | 165 | D | D | T | E | N | A | S | A | Y | A | A | N | A | 23200 |
| 232 | 244 | G | A | M | D | F | T | T | L | Q | A | N | K | S | 23500 |
| 13 | 25 | C | Y | E | N | D | V | N | V | H | H | I | F | F | 24000 |
| 278 | 290 | R | E | Q | M | F | V | R | H | L | F | N | R | A | 24000 |
| 306 | 318 | S | G | S | T | A | N | L | A | S | S | N | Y | F | 25200 |
| 410 | 422 | T | A | D | V | M | T | Y | I | H | S | M | N | S | 25200 |
| 513 | 525 | A | T | P | T | T | S | S | T | S | T | T | A | K | 25500 |
| 56 | 68 | R | T | N | I | Y | Y | H | A | G | T | S | R | L | 26500 |
| 272 | 284 | L | F | F | Y | L | R | R | E | Q | M | F | V | R | 26500 |
| 408 | 420 | T | L | T | A | D | V | M | T | Y | I | H | S | M | 27500 |
| 126 | 138 | W | A | C | V | G | V | E | V | G | R | G | Q | P | 31500 |
| 259 | 271 | D | Y | L | K | M | V | S | E | P | Y | G | D | S | 32000 |
| 317 | 329 | Y | F | P | T | P | S | G | S | M | V | T | S | D | 33500 |
| 373 | 385 | A | I | S | T | S | E | T | T | Y | K | N | T | N | 36000 |
| 497 | 509 | Q | A | G | L | K | A | K | P | K | F | T | L | G | 39000 |
| 47 | 59 | V | V | S | T | D | E | Y | V | A | R | T | N | I | 39500 |
| 415 | 427 | T | Y | I | H | S | M | N | S | T | I | L | E | D | 40200 |
| 77 | 89 | P | I | K | K | P | N | N | N | K | I | L | V | P | 41200 |
| 86 | 98 | I | L | V | P | K | V | S | G | L | Q | Y | R | V | 45000 |
| 214 | 226 | L | E | L | I | N | T | V | I | Q | D | G | D | M | 45000 |
| 324 | 336 | S | M | V | T | S | D | A | Q | I | F | N | K | P | 46000 |
| 2 | 14 | Q | V | T | F | I | Y | I | L | V | I | T | C | Y | 47000 |
| 316 | 328 | N | Y | F | P | T | P | S | G | S | M | V | T | S | 47200 |
| 475 | 487 | V | N | L | K | E | K | F | S | A | D | L | D | Q | 48200 |
| 309 | 321 | T | A | N | L | A | S | S | N | Y | F | P | T | P | 51000 |
| 176 | 188 | D | Y | K | Q | T | Q | L | C | L | I | G | C | K | 54500 |
| 401 | 413 | I | F | Q | L | C | K | I | T | L | T | A | D | V | 54500 |
| 443 | 455 | T | Y | R | F | V | T | Q | A | I | A | C | Q | K | 55500 |
| 50 | 62 | T | D | E | Y | V | A | R | T | N | I | Y | Y | H | 56000 |
| 301 | 313 | L | Y | I | K | G | S | G | S | T | A | N | L | A | 58000 |
| 63 | 75 | A | G | T | S | R | L | L | A | V | G | H | P | Y | 58500 |
| 283 | 295 | V | R | H | L | F | N | R | A | G | T | V | G | E | 65000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 37 | 49 | V | Y | L | P | P | V | P | V | S | K | V | V | S | 66000 |
| 204 | 216 | V | A | V | N | P | G | D | C | P | P | L | E | L | 66000 |
| 276 | 288 | L | R | R | E | Q | M | F | V | R | H | L | F | N | 66000 |
| 29 | 41 | L | W | L | P | S | E | A | T | V | Y | L | P | P | 67200 |
| 502 | 514 | A | K | P | K | F | T | L | G | K | R | K | A | T | 70500 |
| 352 | 364 | G | N | Q | L | F | V | T | V | V | D | T | T | R | 71000 |
| 107 | 119 | N | K | F | G | F | P | D | T | S | F | Y | N | P | 72000 |
| 179 | 191 | Q | T | Q | L | C | L | I | G | C | K | P | P | I | 74000 |
| 327 | 339 | T | S | D | A | Q | I | F | N | K | P | Y | W | L | 74000 |
| 488 | 500 | F | P | L | G | R | K | F | L | L | Q | A | G | L | 74500 |
| 222 | 234 | Q | D | G | D | M | V | H | T | G | F | G | A | M | 76000 |
| 196 | 208 | G | K | G | S | P | C | T | N | V | A | V | N | P | 79000 |
| 494 | 506 | F | L | L | Q | A | G | L | K | A | K | P | K | F | 82000 |
| 91 | 103 | V | S | G | L | Q | Y | R | V | F | R | I | H | L | 86000 |
| 322 | 334 | S | G | S | M | V | T | S | D | A | Q | I | F | N | 89200 |
| 35 | 47 | A | T | V | Y | L | P | P | V | P | V | S | K | V | 92500 |
| 40 | 52 | P | P | V | P | V | S | K | V | V | S | T | D | E | 93500 |
| 82 | 94 | N | N | N | K | I | L | V | P | K | V | S | G | L | 99000 |
| 422 | 434 | S | T | I | L | E | D | W | N | F | G | L | Q | P | 100000 |
| 19 | 31 | N | V | H | H | I | F | F | Q | M | S | L | W | L | 102000 |
| 355 | 367 | L | F | V | T | V | V | D | T | T | R | S | T | N | 102000 |
| 471 | 483 | T | F | W | E | V | N | L | K | E | K | F | S | A | 102000 |
| 360 | 372 | V | D | T | T | R | S | T | N | M | S | L | C | A | 110000 |
| 218 | 230 | N | T | V | I | Q | D | G | D | M | V | H | T | G | 112000 |
| 340 | 352 | Q | R | A | Q | G | H | N | N | G | I | C | W | G | 112000 |
| 266 | 278 | E | P | Y | G | D | S | L | F | F | Y | L | R | R | 115000 |
| 223 | 235 | D | G | D | M | V | H | T | G | F | G | A | M | D | 120000 |
| 7 | 19 | Y | I | L | V | I | T | C | Y | E | N | D | V | N | 125000 |
| 199 | 211 | S | P | C | T | N | V | A | V | N | P | G | D | C | 130000 |
| 358 | 370 | T | V | V | D | T | T | R | S | T | N | M | S | L | 130000 |
| 362 | 374 | T | T | R | S | T | N | M | S | L | C | A | A | I | 130000 |
| 428 | 440 | W | N | F | G | L | Q | P | P | P | G | G | T | L | 130000 |
| 215 | 227 | E | L | I | N | T | V | I | Q | D | G | D | M | V | 132000 |
| 57 | 69 | T | N | I | Y | Y | H | A | G | T | S | R | L | L | 140000 |
| 198 | 210 | G | S | P | C | T | N | V | A | V | N | P | G | D | 142000 |
| 183 | 195 | C | L | I | G | C | K | P | P | I | G | E | H | W | 145000 |
| 60 | 72 | Y | Y | H | A | G | T | S | R | L | L | A | V | G | 150000 |
| 262 | 274 | K | M | V | S | E | P | Y | G | D | S | L | F | F | 152000 |
| 429 | 441 | N | F | G | L | Q | P | P | P | G | G | T | L | E | 155000 |
| 157 | 169 | N | A | S | A | Y | A | A | N | A | G | V | D | N | 162000 |
| 64 | 76 | G | T | S | R | L | L | A | V | G | H | P | Y | F | 165000 |
| 498 | 510 | A | G | L | K | A | K | P | K | F | T | L | G | K | 165000 |
| 140 | 152 | G | V | G | I | S | G | H | P | L | L | N | K | L | 170000 |
| 152 | 164 | L | D | D | T | E | N | A | S | A | Y | A | A | N | 170000 |
| 370 | 382 | L | C | A | A | I | S | T | S | E | T | T | Y | K | 172000 |
| 28 | 40 | S | L | W | L | P | S | E | A | T | V | Y | L | P | 180000 |
| 343 | 355 | Q | G | H | N | N | G | I | C | W | G | N | Q | L | 182000 |
| 118 | 130 | N | P | D | T | Q | R | L | V | W | A | C | V | G | 200000 |
| 314 | 326 | S | S | N | Y | F | P | T | P | S | G | S | M | V | 200000 |
| 381 | 393 | Y | K | N | T | N | F | K | E | Y | L | R | H | G | 202000 |
| 369 | 381 | S | L | C | A | A | I | S | T | S | E | T | T | Y | 205000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 65 | 77 | T | S | R | L | L | A | V | G | H | P | Y | F | P | 210000 |
| 189 | 201 | P | P | I | G | E | H | W | G | K | G | S | P | C | 210000 |
| 93 | 105 | G | L | Q | Y | R | V | F | R | I | H | L | P | D | 220000 |
| 121 | 133 | T | Q | R | L | V | W | A | C | V | G | V | E | V | 220000 |
| 359 | 371 | V | V | D | T | T | R | S | T | N | M | S | L | C | 225000 |
| 346 | 358 | N | N | G | I | C | W | G | N | Q | L | F | V | T | 232000 |
| 33 | 45 | S | E | A | T | V | Y | L | P | P | V | P | V | S | 235000 |
| 53 | 65 | Y | V | A | R | T | N | I | Y | Y | H | A | G | T | 240000 |
| 170 | 182 | R | E | C | I | S | M | D | Y | K | Q | T | Q | L | 240000 |
| 397 | 409 | D | L | Q | F | I | F | Q | L | C | K | I | T | L | 240000 |
| 447 | 459 | V | T | Q | A | I | A | C | Q | K | H | T | P | P | 242000 |
| 286 | 298 | L | F | N | R | A | G | T | V | G | E | N | V | P | 245000 |
| 46 | 58 | K | V | V | S | T | D | E | Y | V | A | R | T | N | 250000 |
| 255 | 267 | C | K | Y | P | D | Y | L | K | M | V | S | E | P | 250000 |
| 120 | 132 | D | T | Q | R | L | V | W | A | C | V | G | V | E | 252000 |
| 30 | 42 | W | L | P | S | E | A | T | V | Y | L | P | P | V | 260000 |
| 117 | 129 | Y | N | P | D | T | Q | R | L | V | W | A | C | V | 270000 |
| 424 | 436 | I | L | E | D | W | N | F | G | L | Q | P | P | P | 270000 |
| 403 | 415 | Q | L | C | K | I | T | L | T | A | D | V | M | T | 275000 |
| 404 | 416 | L | C | K | I | T | L | T | A | D | V | M | T | Y | 335000 |
| 48 | 60 | V | S | T | D | E | Y | V | A | R | T | N | I | Y | 340000 |
| 274 | 286 | F | Y | L | R | R | E | Q | M | F | V | R | H | L | 380000 |
| 495 | 507 | L | L | Q | A | G | L | K | A | K | P | K | F | T | 380000 |
| 260 | 272 | Y | L | K | M | V | S | E | P | Y | G | D | S | L | 382000 |
| 339 | 351 | L | Q | R | A | Q | G | H | N | N | G | I | C | W | 385000 |
| 511 | 523 | R | K | A | T | P | T | T | S | S | T | S | T | T | 390000 |
| 287 | 299 | F | N | R | A | G | T | V | G | E | N | V | P | D | 392000 |
| 356 | 368 | F | V | T | V | V | D | T | T | R | S | T | N | M | 400000 |
| 158 | 170 | A | S | A | Y | A | A | N | A | G | V | D | N | R | 402000 |
| 54 | 66 | V | A | R | T | N | I | Y | Y | H | A | G | T | S | 415000 |
| 269 | 281 | G | D | S | L | F | F | Y | L | R | R | E | Q | M | 430000 |
| 240 | 252 | Q | A | N | K | S | E | V | P | L | D | I | C | T | 440000 |
| 20 | 32 | V | H | H | I | F | F | Q | M | S | L | W | L | P | 442000 |
| 267 | 279 | P | Y | G | D | S | L | F | F | Y | L | R | R | E | 455000 |
| 99 | 111 | F | R | I | H | L | P | D | P | N | K | F | G | F | 462000 |
| 81 | 93 | P | N | N | N | K | I | L | V | P | K | V | S | G | 485000 |
| 62 | 74 | H | A | G | T | S | R | L | L | A | V | G | H | P | 500000 |
| 393 | 405 | G | E | E | Y | D | L | Q | F | I | F | Q | L | C | 512000 |
| 109 | 121 | F | G | F | P | D | T | S | F | Y | N | P | D | T | 555000 |
| 307 | 319 | G | S | T | A | N | L | A | S | S | N | Y | F | P | 555000 |
| 154 | 166 | D | T | E | N | A | S | A | Y | A | A | N | A | G | 562000 |
| 416 | 428 | Y | I | H | S | M | N | S | T | I | L | E | D | W | 572000 |
| 66 | 78 | S | R | L | L | A | V | G | H | P | Y | F | P | I | 575000 |
| 134 | 146 | G | R | G | Q | P | L | G | V | G | I | S | G | H | 575000 |
| 418 | 430 | H | S | M | N | S | T | I | L | E | D | W | N | F | 580000 |
| 334 | 346 | N | K | P | Y | W | L | Q | R | A | Q | G | H | N | 605000 |
| 484 | 496 | D | L | D | Q | F | P | L | G | R | K | F | L | L | 605000 |
| 100 | 112 | R | I | H | L | P | D | P | N | K | F | G | F | P | 625000 |
| 446 | 458 | F | V | T | Q | A | I | A | C | Q | K | H | T | P | 635000 |
| 247 | 259 | P | L | D | I | C | T | S | I | C | K | Y | P | D | 660000 |
| 201 | 213 | C | T | N | V | A | V | N | P | G | D | C | P | P | 670000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 449 | 461 | Q | A | I | A | C | Q | K | H | T | P | P | A | P | 710000 |
| 344 | 356 | G | H | N | N | G | I | C | W | G | N | Q | L | F | 752000 |
| 31 | 43 | L | P | S | E | A | T | V | Y | L | P | P | V | P | 770000 |
| 111 | 123 | F | P | D | T | S | F | Y | N | P | D | T | Q | R | 770000 |
| 348 | 360 | G | I | C | W | G | N | Q | L | F | V | T | V | V | 770000 |
| 105 | 117 | D | P | N | K | F | G | F | P | D | T | S | F | Y | 775000 |
| 112 | 124 | P | D | T | S | F | Y | N | P | D | T | Q | R | L | 782000 |
| 367 | 379 | N | M | S | L | C | A | A | I | S | T | S | E | T | 782000 |
| 83 | 95 | N | N | K | I | L | V | P | K | V | S | G | L | Q | 802000 |
| 220 | 232 | V | I | Q | D | G | D | M | V | H | T | G | F | G | 810000 |
| 321 | 333 | P | S | G | S | M | V | T | S | D | A | Q | I | F | 810000 |
| 439 | 451 | T | L | E | D | T | Y | R | F | V | T | Q | A | I | 842000 |
| 489 | 501 | P | L | G | R | K | F | L | L | Q | A | G | L | K | 905000 |
| 282 | 294 | F | V | R | H | L | F | N | R | A | G | T | V | G | 915000 |
| 354 | 366 | Q | L | F | V | T | V | V | D | T | T | R | S | T | 980000 |
| 26 | 38 | Q | M | S | L | W | L | P | S | E | A | T | V | Y | 995000 |
| 4 | 16 | T | F | I | Y | I | L | V | I | T | C | Y | E | N | 1000000 |
| 10 | 22 | V | I | T | C | Y | E | N | D | V | N | V | H | H | 1000000 |
| 11 | 23 | I | T | C | Y | E | N | D | V | N | V | H | H | I | 1000000 |
| 14 | 26 | Y | E | N | D | V | N | V | H | H | I | F | F | Q | 1000000 |
| 15 | 27 | E | N | D | V | N | V | H | H | I | F | F | Q | M | 1000000 |
| 17 | 29 | D | V | N | V | H | H | I | F | F | Q | M | S | L | 1000000 |
| 24 | 36 | F | F | Q | M | S | L | W | L | P | S | E | A | T | 1000000 |
| 32 | 44 | P | S | E | A | T | V | Y | L | P | P | V | P | V | 1000000 |
| 38 | 50 | Y | L | P | P | V | P | V | S | K | V | V | S | T | 1000000 |
| 39 | 51 | L | P | P | V | P | V | S | K | V | V | S | T | D | 1000000 |
| 41 | 53 | P | V | P | V | S | K | V | V | S | T | D | E | Y | 1000000 |
| 42 | 54 | V | P | V | S | K | V | V | S | T | D | E | Y | V | 1000000 |
| 43 | 55 | P | V | S | K | V | V | S | T | D | E | Y | V | A | 1000000 |
| 44 | 56 | V | S | K | V | V | S | T | D | E | Y | V | A | R | 1000000 |
| 49 | 61 | S | T | D | E | Y | V | A | R | T | N | I | Y | Y | 1000000 |
| 55 | 67 | A | R | T | N | I | Y | Y | H | A | G | T | S | R | 1000000 |
| 61 | 73 | Y | H | A | G | T | S | R | L | L | A | V | G | H | 1000000 |
| 68 | 80 | L | L | A | V | G | H | P | Y | F | P | I | K | K | 1000000 |
| 69 | 81 | L | A | V | G | H | P | Y | F | P | I | K | K | P | 1000000 |
| 70 | 82 | A | V | G | H | P | Y | F | P | I | K | K | P | N | 1000000 |
| 71 | 83 | V | G | H | P | Y | F | P | I | K | K | P | N | N | 1000000 |
| 72 | 84 | G | H | P | Y | F | P | I | K | K | P | N | N | N | 1000000 |
| 74 | 86 | P | Y | F | P | I | K | K | P | N | N | N | K | I | 1000000 |
| 75 | 87 | Y | F | P | I | K | K | P | N | N | N | K | I | L | 1000000 |
| 78 | 90 | I | K | K | P | N | N | N | K | I | L | V | P | K | 1000000 |
| 79 | 91 | K | K | P | N | N | N | K | I | L | V | P | K | V | 1000000 |
| 80 | 92 | K | P | N | N | N | K | I | L | V | P | K | V | S | 1000000 |
| 87 | 99 | L | V | P | K | V | S | G | L | Q | Y | R | V | F | 1000000 |
| 90 | 102 | K | V | S | G | L | Q | Y | R | V | F | R | I | H | 1000000 |
| 95 | 107 | Q | Y | R | V | F | R | I | H | L | P | D | P | N | 1000000 |
| 96 | 108 | Y | R | V | F | R | I | H | L | P | D | P | N | K | 1000000 |
| 98 | 110 | V | F | R | I | H | L | P | D | P | N | K | F | G | 1000000 |
| 101 | 113 | I | H | L | P | D | P | N | K | F | G | F | P | D | 1000000 |
| 102 | 114 | H | L | P | D | P | N | K | F | G | F | P | D | T | 1000000 |
| 103 | 115 | L | P | D | P | N | K | F | G | F | P | D | T | S | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 104 | 116 | P | D | P | N | K | F | G | F | P | D | T | S | F | 1000000 |
| 106 | 118 | P | N | K | F | G | F | P | D | T | S | F | Y | N | 1000000 |
| 108 | 120 | K | F | G | F | P | D | T | S | F | Y | N | P | D | 1000000 |
| 110 | 122 | G | F | P | D | T | S | F | Y | N | P | D | T | Q | 1000000 |
| 113 | 125 | D | T | S | F | Y | N | P | D | T | Q | R | L | V | 1000000 |
| 116 | 128 | F | Y | N | P | D | T | Q | R | L | V | W | A | C | 1000000 |
| 119 | 131 | P | D | T | Q | R | L | V | W | A | C | V | G | V | 1000000 |
| 125 | 137 | V | W | A | C | V | G | V | E | V | G | R | G | Q | 1000000 |
| 128 | 140 | C | V | G | V | E | V | G | R | G | Q | P | L | G | 1000000 |
| 130 | 142 | G | V | E | V | G | R | G | Q | P | L | G | V | G | 1000000 |
| 132 | 144 | E | V | G | R | G | Q | P | L | G | V | G | I | S | 1000000 |
| 133 | 145 | V | G | R | G | Q | P | L | G | V | G | I | S | G | 1000000 |
| 135 | 147 | R | G | Q | P | L | G | V | G | I | S | G | H | P | 1000000 |
| 136 | 148 | G | Q | P | L | G | V | G | I | S | G | H | P | L | 1000000 |
| 139 | 151 | L | G | V | G | I | S | G | H | P | L | L | N | K | 1000000 |
| 142 | 154 | G | I | S | G | H | P | L | L | N | K | L | D | D | 1000000 |
| 143 | 155 | I | S | G | H | P | L | L | N | K | L | D | D | T | 1000000 |
| 144 | 156 | S | G | H | P | L | L | N | K | L | D | D | T | E | 1000000 |
| 145 | 157 | G | H | P | L | L | N | K | L | D | D | T | E | N | 1000000 |
| 146 | 158 | H | P | L | L | N | K | L | D | D | T | E | N | A | 1000000 |
| 147 | 159 | P | L | L | N | K | L | D | D | T | E | N | A | S | 1000000 |
| 148 | 160 | L | L | N | K | L | D | D | T | E | N | A | S | A | 1000000 |
| 149 | 161 | L | N | K | L | D | D | T | E | N | A | S | A | Y | 1000000 |
| 151 | 163 | K | L | D | D | T | E | N | A | S | A | Y | A | A | 1000000 |
| 160 | 172 | A | Y | A | A | N | A | G | V | D | N | R | E | C | 1000000 |
| 161 | 173 | Y | A | A | N | A | G | V | D | N | R | E | C | I | 1000000 |
| 162 | 174 | A | A | N | A | G | V | D | N | R | E | C | I | S | 1000000 |
| 163 | 175 | A | N | A | G | V | D | N | R | E | C | I | S | M | 1000000 |
| 164 | 176 | N | A | G | V | D | N | R | E | C | I | S | M | D | 1000000 |
| 166 | 178 | G | V | D | N | R | E | C | I | S | M | D | Y | K | 1000000 |
| 167 | 179 | V | D | N | R | E | C | I | S | M | D | Y | K | Q | 1000000 |
| 168 | 180 | D | N | R | E | C | I | S | M | D | Y | K | Q | T | 1000000 |
| 169 | 181 | N | R | E | C | I | S | M | D | Y | K | Q | T | Q | 1000000 |
| 172 | 184 | C | I | S | M | D | Y | K | Q | T | Q | L | C | L | 1000000 |
| 174 | 186 | S | M | D | Y | K | Q | T | Q | L | C | L | I | G | 1000000 |
| 177 | 189 | Y | K | Q | T | Q | L | C | L | I | G | C | K | P | 1000000 |
| 178 | 190 | K | Q | T | Q | L | C | L | I | G | C | K | P | P | 1000000 |
| 181 | 193 | Q | L | C | L | I | G | C | K | P | P | I | G | E | 1000000 |
| 184 | 196 | L | I | G | C | K | P | P | I | G | E | H | W | G | 1000000 |
| 185 | 197 | I | G | C | K | P | P | I | G | E | H | W | G | K | 1000000 |
| 186 | 198 | G | C | K | P | P | I | G | E | H | W | G | K | G | 1000000 |
| 187 | 199 | C | K | P | P | I | G | E | H | W | G | K | G | S | 1000000 |
| 188 | 200 | K | P | P | I | G | E | H | W | G | K | G | S | P | 1000000 |
| 190 | 202 | P | I | G | E | H | W | G | K | G | S | P | C | T | 1000000 |
| 191 | 203 | I | G | E | H | W | G | K | G | S | P | C | T | N | 1000000 |
| 192 | 204 | G | E | H | W | G | K | G | S | P | C | T | N | V | 1000000 |
| 194 | 206 | H | W | G | K | G | S | P | C | T | N | V | A | V | 1000000 |
| 195 | 207 | W | G | K | G | S | P | C | T | N | V | A | V | N | 1000000 |
| 197 | 209 | K | G | S | P | C | T | N | V | A | V | N | P | G | 1000000 |
| 200 | 212 | P | C | T | N | V | A | V | N | P | G | D | C | P | 1000000 |
| 203 | 215 | N | V | A | V | N | P | G | D | C | P | P | L | E | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 205 | 217 | A | V | N | P | G | D | C | P | P | L | E | L | I | 1000000 |
| 206 | 218 | V | N | P | G | D | C | P | P | L | E | L | I | N | 1000000 |
| 207 | 219 | N | P | G | D | C | P | P | L | E | L | I | N | T | 1000000 |
| 208 | 220 | P | G | D | C | P | P | L | E | L | I | N | T | V | 1000000 |
| 209 | 221 | G | D | C | P | P | L | E | L | I | N | T | V | I | 1000000 |
| 210 | 222 | D | C | P | P | L | E | L | I | N | T | V | I | Q | 1000000 |
| 211 | 223 | C | P | P | L | E | L | I | N | T | V | I | Q | D | 1000000 |
| 213 | 225 | P | L | E | L | I | N | T | V | I | Q | D | G | D | 1000000 |
| 216 | 228 | L | I | N | T | V | I | Q | D | G | D | M | V | H | 1000000 |
| 217 | 229 | I | N | T | V | I | Q | D | G | D | M | V | H | T | 1000000 |
| 221 | 233 | I | Q | D | G | D | M | V | H | T | G | F | G | A | 1000000 |
| 226 | 238 | M | V | H | T | G | F | G | A | M | D | F | T | T | 1000000 |
| 227 | 239 | V | H | T | G | F | G | A | M | D | F | T | T | L | 1000000 |
| 228 | 240 | H | T | G | F | G | A | M | D | F | T | T | L | Q | 1000000 |
| 229 | 241 | T | G | F | G | A | M | D | F | T | T | L | Q | A | 1000000 |
| 230 | 242 | G | F | G | A | M | D | F | T | T | L | Q | A | N | 1000000 |
| 233 | 245 | A | M | D | F | T | T | L | Q | A | N | K | S | E | 1000000 |
| 235 | 247 | D | F | T | T | L | Q | A | N | K | S | E | V | P | 1000000 |
| 236 | 248 | F | T | T | L | Q | A | N | K | S | E | V | P | L | 1000000 |
| 238 | 250 | T | L | Q | A | N | K | S | E | V | P | L | D | I | 1000000 |
| 239 | 251 | L | Q | A | N | K | S | E | V | P | L | D | I | C | 1000000 |
| 241 | 253 | A | N | K | S | E | V | P | L | D | I | C | T | S | 1000000 |
| 242 | 254 | N | K | S | E | V | P | L | D | I | C | T | S | I | 1000000 |
| 243 | 255 | K | S | E | V | P | L | D | I | C | T | S | I | C | 1000000 |
| 244 | 256 | S | E | V | P | L | D | I | C | T | S | I | C | K | 1000000 |
| 245 | 257 | E | V | P | L | D | I | C | T | S | I | C | K | Y | 1000000 |
| 249 | 261 | D | I | C | T | S | I | C | K | Y | P | D | Y | L | 1000000 |
| 250 | 262 | I | C | T | S | I | C | K | Y | P | D | Y | L | K | 1000000 |
| 251 | 263 | C | T | S | I | C | K | Y | P | D | Y | L | K | M | 1000000 |
| 253 | 265 | S | I | C | K | Y | P | D | Y | L | K | M | V | S | 1000000 |
| 254 | 266 | I | C | K | Y | P | D | Y | L | K | M | V | S | E | 1000000 |
| 256 | 268 | K | Y | P | D | Y | L | K | M | V | S | E | P | Y | 1000000 |
| 257 | 269 | Y | P | D | Y | L | K | M | V | S | E | P | Y | G | 1000000 |
| 263 | 275 | M | V | S | E | P | Y | G | D | S | L | F | F | Y | 1000000 |
| 264 | 276 | V | S | E | P | Y | G | D | S | L | F | F | Y | L | 1000000 |
| 265 | 277 | S | E | P | Y | G | D | S | L | F | F | Y | L | R | 1000000 |
| 268 | 280 | Y | G | D | S | L | F | F | Y | L | R | R | E | Q | 1000000 |
| 275 | 287 | Y | L | R | R | E | Q | M | F | V | R | H | L | F | 1000000 |
| 277 | 289 | R | R | E | Q | M | F | V | R | H | L | F | N | R | 1000000 |
| 279 | 291 | E | Q | M | F | V | R | H | L | F | N | R | A | G | 1000000 |
| 284 | 296 | R | H | L | F | N | R | A | G | T | V | G | E | N | 1000000 |
| 288 | 300 | N | R | A | G | T | V | G | E | N | V | P | D | D | 1000000 |
| 289 | 301 | R | A | G | T | V | G | E | N | V | P | D | D | L | 1000000 |
| 290 | 302 | A | G | T | V | G | E | N | V | P | D | D | L | Y | 1000000 |
| 291 | 303 | G | T | V | G | E | N | V | P | D | D | L | Y | I | 1000000 |
| 292 | 304 | T | V | G | E | N | V | P | D | D | L | Y | I | K | 1000000 |
| 293 | 305 | V | G | E | N | V | P | D | D | L | Y | I | K | G | 1000000 |
| 294 | 306 | G | E | N | V | P | D | D | L | Y | I | K | G | S | 1000000 |
| 295 | 307 | E | N | V | P | D | D | L | Y | I | K | G | S | G | 1000000 |
| 296 | 308 | N | V | P | D | D | L | Y | I | K | G | S | G | S | 1000000 |
| 297 | 309 | V | P | D | D | L | Y | I | K | G | S | G | S | T | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 298 | 310 | P | D | D | L | Y | I | K | G | S | G | S | T | A | 1000000 |
| 302 | 314 | Y | I | K | G | S | G | S | T | A | N | L | A | S | 1000000 |
| 304 | 316 | K | G | S | G | S | T | A | N | L | A | S | S | N | 1000000 |
| 305 | 317 | G | S | G | S | T | A | N | L | A | S | S | N | Y | 1000000 |
| 308 | 320 | S | T | A | N | L | A | S | S | N | Y | F | P | T | 1000000 |
| 311 | 323 | N | L | A | S | S | N | Y | F | P | T | P | S | G | 1000000 |
| 312 | 324 | L | A | S | S | N | Y | F | P | T | P | S | G | S | 1000000 |
| 313 | 325 | A | S | S | N | Y | F | P | T | P | S | G | S | M | 1000000 |
| 318 | 330 | F | P | T | P | S | G | S | M | V | T | S | D | A | 1000000 |
| 319 | 331 | P | T | P | S | G | S | M | V | T | S | D | A | Q | 1000000 |
| 320 | 332 | T | P | S | G | S | M | V | T | S | D | A | Q | I | 1000000 |
| 325 | 337 | M | V | T | S | D | A | Q | I | F | N | K | P | Y | 1000000 |
| 326 | 338 | V | T | S | D | A | Q | I | F | N | K | P | Y | W | 1000000 |
| 328 | 340 | S | D | A | Q | I | F | N | K | P | Y | W | L | Q | 1000000 |
| 332 | 344 | I | F | N | K | P | Y | W | L | Q | R | A | Q | G | 1000000 |
| 333 | 345 | F | N | K | P | Y | W | L | Q | R | A | Q | G | H | 1000000 |
| 338 | 350 | W | L | Q | R | A | Q | G | H | N | N | G | I | C | 1000000 |
| 341 | 353 | R | A | Q | G | H | N | N | G | I | C | W | G | N | 1000000 |
| 342 | 354 | A | Q | G | H | N | N | G | I | C | W | G | N | Q | 1000000 |
| 345 | 357 | H | N | N | G | I | C | W | G | N | Q | L | F | V | 1000000 |
| 350 | 362 | C | W | G | N | Q | L | F | V | T | V | V | D | T | 1000000 |
| 351 | 363 | W | G | N | Q | L | F | V | T | V | V | D | T | T | 1000000 |
| 361 | 373 | D | T | T | R | S | T | N | M | S | L | C | A | A | 1000000 |
| 363 | 375 | T | R | S | T | N | M | S | L | C | A | A | I | S | 1000000 |
| 368 | 380 | M | S | L | C | A | A | I | S | T | S | E | T | T | 1000000 |
| 371 | 383 | C | A | A | I | S | T | S | E | T | T | Y | K | N | 1000000 |
| 372 | 384 | A | A | I | S | T | S | E | T | T | Y | K | N | T | 1000000 |
| 374 | 386 | I | S | T | S | E | T | T | Y | K | N | T | N | F | 1000000 |
| 375 | 387 | S | T | S | E | T | T | Y | K | N | T | N | F | K | 1000000 |
| 376 | 388 | T | S | E | T | T | Y | K | N | T | N | F | K | E | 1000000 |
| 377 | 389 | S | E | T | T | Y | K | N | T | N | F | K | E | Y | 1000000 |
| 378 | 390 | E | T | T | Y | K | N | T | N | F | K | E | Y | L | 1000000 |
| 380 | 392 | T | Y | K | N | T | N | F | K | E | Y | L | R | H | 1000000 |
| 382 | 394 | K | N | T | N | F | K | E | Y | L | R | H | G | E | 1000000 |
| 383 | 395 | N | T | N | F | K | E | Y | L | R | H | G | E | E | 1000000 |
| 385 | 397 | N | F | K | E | Y | L | R | H | G | E | E | Y | D | 1000000 |
| 386 | 398 | F | K | E | Y | L | R | H | G | E | E | Y | D | L | 1000000 |
| 389 | 401 | Y | L | R | H | G | E | E | Y | D | L | Q | F | I | 1000000 |
| 390 | 402 | L | R | H | G | E | E | Y | D | L | Q | F | I | F | 1000000 |
| 391 | 403 | R | H | G | E | E | Y | D | L | Q | F | I | F | Q | 1000000 |
| 392 | 404 | H | G | E | E | Y | D | L | Q | F | I | F | Q | L | 1000000 |
| 395 | 407 | E | Y | D | L | Q | F | I | F | Q | L | C | K | I | 1000000 |
| 402 | 414 | F | Q | L | C | K | I | T | L | T | A | D | V | M | 1000000 |
| 406 | 418 | K | I | T | L | T | A | D | V | M | T | Y | I | H | 1000000 |
| 407 | 419 | I | T | L | T | A | D | V | M | T | Y | I | H | S | 1000000 |
| 409 | 421 | L | T | A | D | V | M | T | Y | I | H | S | M | N | 1000000 |
| 413 | 425 | V | M | T | Y | I | H | S | M | N | S | T | I | L | 1000000 |
| 417 | 429 | I | H | S | M | N | S | T | I | L | E | D | W | N | 1000000 |
| 419 | 431 | S | M | N | S | T | I | L | E | D | W | N | F | G | 1000000 |
| 420 | 432 | M | N | S | T | I | L | E | D | W | N | F | G | L | 1000000 |
| 421 | 433 | N | S | T | I | L | E | D | W | N | F | G | L | Q | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 425 | 437 | L | E | D | W | N | F | G | L | Q | P | P | P | G | 1000000 |
| 427 | 439 | D | W | N | F | G | L | Q | P | P | P | G | G | T | 1000000 |
| 431 | 443 | G | L | Q | P | P | P | G | G | T | L | E | D | T | 1000000 |
| 432 | 444 | L | Q | P | P | P | G | G | T | L | E | D | T | Y | 1000000 |
| 433 | 445 | Q | P | P | P | G | G | T | L | E | D | T | Y | R | 1000000 |
| 434 | 446 | P | P | P | G | G | T | L | E | D | T | Y | R | F | 1000000 |
| 435 | 447 | P | P | G | G | T | L | E | D | T | Y | R | F | V | 1000000 |
| 436 | 448 | P | G | G | T | L | E | D | T | Y | R | F | V | T | 1000000 |
| 437 | 449 | G | G | T | L | E | D | T | Y | R | F | V | T | Q | 1000000 |
| 440 | 452 | L | E | D | T | Y | R | F | V | T | Q | A | I | A | 1000000 |
| 448 | 460 | T | Q | A | I | A | C | Q | K | H | T | P | P | A | 1000000 |
| 450 | 462 | A | I | A | C | Q | K | H | T | P | P | A | P | K | 1000000 |
| 451 | 463 | I | A | C | Q | K | H | T | P | P | A | P | K | E | 1000000 |
| 452 | 464 | A | C | Q | K | H | T | P | P | A | P | K | E | D | 1000000 |
| 453 | 465 | C | Q | K | H | T | P | P | A | P | K | E | D | D | 1000000 |
| 454 | 466 | Q | K | H | T | P | P | A | P | K | E | D | D | P | 1000000 |
| 455 | 467 | K | H | T | P | P | A | P | K | E | D | D | P | L | 1000000 |
| 456 | 468 | H | T | P | P | A | P | K | E | D | D | P | L | K | 1000000 |
| 457 | 469 | T | P | P | A | P | K | E | D | D | P | L | K | K | 1000000 |
| 458 | 470 | P | P | A | P | K | E | D | D | P | L | K | K | Y | 1000000 |
| 459 | 471 | P | A | P | K | E | D | D | P | L | K | K | Y | T | 1000000 |
| 460 | 472 | A | P | K | E | D | D | P | L | K | K | Y | T | F | 1000000 |
| 461 | 473 | P | K | E | D | D | P | L | K | K | Y | T | F | W | 1000000 |
| 462 | 474 | K | E | D | D | P | L | K | K | Y | T | F | W | E | 1000000 |
| 463 | 475 | E | D | D | P | L | K | K | Y | T | F | W | E | V | 1000000 |
| 464 | 476 | D | D | P | L | K | K | Y | T | F | W | E | V | N | 1000000 |
| 466 | 478 | P | L | K | K | Y | T | F | W | E | V | N | L | K | 1000000 |
| 467 | 479 | L | K | K | Y | T | F | W | E | V | N | L | K | E | 1000000 |
| 469 | 481 | K | Y | T | F | W | E | V | N | L | K | E | K | F | 1000000 |
| 472 | 484 | F | W | E | V | N | L | K | E | K | F | S | A | D | 1000000 |
| 473 | 485 | W | E | V | N | L | K | E | K | F | S | A | D | L | 1000000 |
| 474 | 486 | E | V | N | L | K | E | K | F | S | A | D | L | D | 1000000 |
| 476 | 488 | N | L | K | E | K | F | S | A | D | L | D | Q | F | 1000000 |
| 477 | 489 | L | K | E | K | F | S | A | D | L | D | Q | F | P | 1000000 |
| 478 | 490 | K | E | K | F | S | A | D | L | D | Q | F | P | L | 1000000 |
| 480 | 492 | K | F | S | A | D | L | D | Q | F | P | L | G | R | 1000000 |
| 481 | 493 | F | S | A | D | L | D | Q | F | P | L | G | R | K | 1000000 |
| 482 | 494 | S | A | D | L | D | Q | F | P | L | G | R | K | F | 1000000 |
| 485 | 497 | L | D | Q | F | P | L | G | R | K | F | L | L | Q | 1000000 |
| 486 | 498 | D | Q | F | P | L | G | R | K | F | L | L | Q | A | 1000000 |
| 487 | 499 | Q | F | P | L | G | R | K | F | L | L | Q | A | G | 1000000 |
| 490 | 502 | L | G | R | K | F | L | L | Q | A | G | L | K | A | 1000000 |
| 491 | 503 | G | R | K | F | L | L | Q | A | G | L | K | A | K | 1000000 |
| 496 | 508 | L | Q | A | G | L | K | A | K | P | K | F | T | L | 1000000 |
| 499 | 511 | G | L | K | A | K | P | K | F | T | L | G | K | R | 1000000 |
| 500 | 512 | L | K | A | K | P | K | F | T | L | G | K | R | K | 1000000 |
| 501 | 513 | K | A | K | P | K | F | T | L | G | K | R | K | A | 1000000 |
| 503 | 515 | K | P | K | F | T | L | G | K | R | K | A | T | P | 1000000 |
| 505 | 517 | K | F | T | L | G | K | R | K | A | T | P | T | T | 1000000 |
| 506 | 518 | F | T | L | G | K | R | K | A | T | P | T | T | S | 1000000 |
| 507 | 519 | T | L | G | K | R | K | A | T | P | T | T | S | S | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 508 | 520 | L | G | K | R | K | A | T | P | T | T | S | S | T | 1000000 |
| 509 | 521 | G | K | R | K | A | T | P | T | T | S | S | T | S | 1000000 |
| 510 | 522 | K | R | K | A | T | P | T | T | S | S | T | S | T | 1000000 |
| 512 | 524 | K | A | T | P | T | T | S | S | T | S | T | T | A | 1000000 |
| 514 | 526 | T | P | T | T | S | S | T | S | T | T | A | K | R | 1000000 |
| 515 | 527 | P | T | T | S | S | T | S | T | T | A | K | R | K | 1000000 |
| 516 | 528 | T | T | S | S | T | S | T | T | A | K | R | K | K | 1000000 |
| 517 | 529 | T | S | S | T | S | T | T | A | K | R | K | K | R | 1000000 |
| 518 | 530 | S | S | T | S | T | T | A | K | R | K | K | R | K | 1000000 |
| 519 | 531 | S | T | S | T | T | A | K | R | K | K | R | K | L | 1000000 |

| | | | | | | | | | | | | | | | IC50 DRB1*0401 (nM) |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------------------|
| 442 | 454 | D | T | Y | R | F | V | T | Q | A | I | A | C | Q | 1.15 |
| 492 | 504 | R | K | F | L | L | Q | A | G | L | K | A | K | P | 3 |
| 94 | 106 | L | Q | Y | R | V | F | R | I | H | L | P | D | P | 4.3 |
| 3 | 15 | V | T | F | I | Y | I | L | V | I | T | C | Y | E | 10.5 |
| 124 | 136 | L | V | W | A | C | V | G | V | E | V | G | R | G | 21 |
| 412 | 424 | D | V | M | T | Y | I | H | S | M | N | S | T | I | 27.5 |
| 5 | 17 | F | I | Y | I | L | V | I | T | C | Y | E | N | D | 28.2 |
| 159 | 171 | S | A | Y | A | A | N | A | G | V | D | N | R | E | 31.5 |
| 444 | 456 | Y | R | F | V | T | Q | A | I | A | C | Q | K | H | 40 |
| 6 | 18 | I | Y | I | L | V | I | T | C | Y | E | N | D | V | 71.5 |
| 23 | 35 | I | F | F | Q | M | S | L | W | L | P | S | E | A | 88.2 |
| 394 | 406 | E | E | Y | D | L | Q | F | I | F | Q | L | C | K | 92 |
| 400 | 412 | F | I | F | Q | L | C | K | I | T | L | T | A | D | 96.5 |
| 28 | 40 | S | L | W | L | P | S | E | A | T | V | Y | L | P | 102 |
| 58 | 70 | N | I | Y | Y | H | A | G | T | S | R | L | L | A | 105 |
| 97 | 109 | R | V | F | R | I | H | L | P | D | P | N | K | F | 105 |
| 9 | 21 | L | V | I | T | C | Y | E | N | D | V | N | V | H | 110 |
| 414 | 426 | M | T | Y | I | H | S | M | N | S | T | I | L | E | 110 |
| 415 | 427 | T | Y | I | H | S | M | N | S | T | I | L | E | D | 120 |
| 301 | 313 | L | Y | I | K | G | S | G | S | T | A | N | L | A | 135 |
| 45 | 57 | S | K | V | V | S | T | D | E | Y | V | A | R | T | 145 |
| 1 | 13 | M | Q | V | T | F | I | Y | I | L | V | I | T | C | 162 |
| 21 | 33 | H | H | I | F | F | Q | M | S | L | W | L | P | S | 165 |
| 25 | 37 | F | Q | M | S | L | W | L | P | S | E | A | T | V | 200 |
| 422 | 434 | S | T | I | L | E | D | W | N | F | G | L | Q | P | 242 |
| 281 | 293 | M | F | V | R | H | L | F | N | R | A | G | T | V | 245 |
| 182 | 194 | L | C | L | I | G | C | K | P | P | I | G | E | H | 265 |
| 428 | 440 | W | N | F | G | L | Q | P | P | P | G | G | T | L | 280 |
| 51 | 63 | D | E | Y | V | A | R | T | N | I | Y | Y | H | A | 310 |
| 234 | 246 | M | D | F | T | T | L | Q | A | N | K | S | E | V | 310 |
| 237 | 249 | T | T | L | Q | A | N | K | S | E | V | P | L | D | 310 |
| 259 | 271 | D | Y | L | K | M | V | S | E | P | Y | G | D | S | 322 |
| 398 | 410 | L | Q | F | I | F | Q | L | C | K | I | T | L | T | 322 |
| 355 | 367 | L | F | V | T | V | V | D | T | T | R | S | T | N | 330 |
| 258 | 270 | P | D | Y | L | K | M | V | S | E | P | Y | G | D | 342 |
| 449 | 461 | Q | A | I | A | C | Q | K | H | T | P | P | A | P | 352 |
| 300 | 312 | D | L | Y | I | K | G | S | G | S | T | A | N | L | 425 |
| 4 | 16 | T | F | I | Y | I | L | V | I | T | C | Y | E | N | 430 |
| 261 | 273 | L | K | M | V | S | E | P | Y | G | D | S | L | F | 445 |
| 324 | 336 | S | M | V | T | S | D | A | Q | I | F | N | K | P | 505 |
| 22 | 34 | H | I | F | F | Q | M | S | L | W | L | P | S | E | 540 |
| 52 | 64 | E | Y | V | A | R | T | N | I | Y | Y | H | A | G | 550 |
| 426 | 438 | E | D | W | N | F | G | L | Q | P | P | P | G | G | 612 |
| 123 | 135 | R | L | V | W | A | C | V | G | V | E | V | G | R | 650 |
| 366 | 378 | T | N | M | S | L | C | A | A | I | S | T | S | E | 710 |
| 280 | 292 | Q | M | F | V | R | H | L | F | N | R | A | G | T | 732 |
| 468 | 480 | K | K | Y | T | F | W | E | V | N | L | K | E | K | 745 |
| 372 | 384 | A | A | I | S | T | S | E | T | T | Y | K | N | T | 815 |
| 115 | 127 | S | F | Y | N | P | D | T | Q | R | L | V | W | A | 835 |
| 405 | 417 | C | K | I | T | L | T | A | D | V | M | T | Y | I | 880 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|------|
| 59 | 71 | I | Y | Y | H | A | G | T | S | R | L | L | A | V | 922 |
| 271 | 283 | S | L | F | F | Y | L | R | R | E | Q | M | F | V | 1050 |
| 273 | 285 | F | F | Y | L | R | R | E | Q | M | F | V | R | H | 1420 |
| 337 | 349 | Y | W | L | Q | R | A | Q | G | H | N | N | G | I | 1420 |
| 16 | 28 | N | D | V | N | V | H | H | I | F | F | Q | M | S | 1450 |
| 141 | 153 | V | G | I | S | G | H | P | L | L | N | K | L | D | 1500 |
| 335 | 347 | K | P | Y | W | L | Q | R | A | Q | G | H | N | N | 1500 |
| 175 | 187 | M | D | Y | K | Q | T | Q | L | C | L | I | G | C | 1700 |
| 421 | 433 | N | S | T | I | L | E | D | W | N | F | G | L | Q | 1850 |
| 388 | 400 | E | Y | L | R | H | G | E | E | Y | D | L | Q | F | 1900 |
| 150 | 162 | N | K | L | D | D | T | E | N | A | S | A | Y | A | 2300 |
| 57 | 69 | T | N | I | Y | Y | H | A | G | T | S | R | L | L | 2500 |
| 53 | 65 | Y | V | A | R | T | N | I | Y | Y | H | A | G | T | 2650 |
| 347 | 359 | N | G | I | C | W | G | N | Q | L | F | V | T | V | 2700 |
| 67 | 79 | R | L | L | A | V | G | H | P | Y | F | P | I | K | 2720 |
| 315 | 327 | S | N | Y | F | P | T | P | S | G | S | M | V | T | 2800 |
| 361 | 373 | D | T | T | R | S | T | N | M | S | L | C | A | A | 3200 |
| 407 | 419 | I | T | L | T | A | D | V | M | T | Y | I | H | S | 3320 |
| 204 | 216 | V | A | V | N | P | G | D | C | P | P | L | E | L | 3400 |
| 218 | 230 | N | T | V | I | Q | D | G | D | M | V | H | T | G | 3520 |
| 445 | 457 | R | F | V | T | Q | A | I | A | C | Q | K | H | T | 3600 |
| 12 | 24 | T | C | Y | E | N | D | V | N | V | H | H | I | F | 3700 |
| 96 | 108 | Y | R | V | F | R | I | H | L | P | D | P | N | K | 3850 |
| 46 | 58 | K | V | V | S | T | D | E | Y | V | A | R | T | N | 4020 |
| 285 | 297 | H | L | F | N | R | A | G | T | V | G | E | N | V | 4020 |
| 202 | 214 | T | N | V | A | V | N | P | G | D | C | P | P | L | 4100 |
| 8 | 20 | I | L | V | I | T | C | Y | E | N | D | V | N | V | 4250 |
| 418 | 430 | H | S | M | N | S | T | I | L | E | D | W | N | F | 4300 |
| 2 | 14 | Q | V | T | F | I | Y | I | L | V | I | T | C | Y | 4350 |
| 229 | 241 | T | G | F | G | A | M | D | F | T | T | L | Q | A | 4350 |
| 66 | 78 | S | R | L | L | A | V | G | H | P | Y | F | P | I | 4500 |
| 362 | 374 | T | T | R | S | T | N | M | S | L | C | A | A | I | 4700 |
| 291 | 303 | G | T | V | G | E | N | V | P | D | D | L | Y | I | 4720 |
| 219 | 231 | T | V | I | Q | D | G | D | M | V | H | T | G | F | 4820 |
| 274 | 286 | F | Y | L | R | R | E | Q | M | F | V | R | H | L | 4820 |
| 139 | 151 | L | G | V | G | I | S | G | H | P | L | L | N | K | 5000 |
| 479 | 491 | E | K | F | S | A | D | L | D | Q | F | P | L | G | 5150 |
| 387 | 399 | K | E | Y | L | R | H | G | E | E | Y | D | L | Q | 5900 |
| 310 | 322 | A | N | L | A | S | S | N | Y | F | P | T | P | S | 6020 |
| 408 | 420 | T | L | T | A | D | V | M | T | Y | I | H | S | M | 6200 |
| 120 | 132 | D | T | Q | R | L | V | W | A | C | V | G | V | E | 7220 |
| 225 | 237 | D | M | V | H | T | G | F | G | A | M | D | F | T | 7300 |
| 35 | 47 | A | T | V | Y | L | P | P | V | P | V | S | K | V | 7450 |
| 214 | 226 | L | E | L | I | N | T | V | I | Q | D | G | D | M | 7900 |
| 73 | 85 | H | P | Y | F | P | I | K | K | P | N | N | N | K | 7950 |
| 164 | 176 | N | A | G | V | D | N | R | E | C | I | S | M | D | 8120 |
| 354 | 366 | Q | L | F | V | T | V | V | D | T | T | R | S | T | 8700 |
| 329 | 341 | D | A | Q | I | F | N | K | P | Y | W | L | Q | R | 8800 |
| 231 | 243 | F | G | A | M | D | F | T | T | L | Q | A | N | K | 9200 |
| 309 | 321 | T | A | N | L | A | S | S | N | Y | F | P | T | P | 9550 |
| 353 | 365 | N | Q | L | F | V | T | V | V | D | T | T | R | S | 9950 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|-------|
| 342 | 354 | A | Q | G | H | N | N | G | I | C | W | G | N | Q | 11000 |
| 138 | 150 | P | L | G | V | G | I | S | G | H | P | L | L | N | 11200 |
| 336 | 348 | P | Y | W | L | Q | R | A | Q | G | H | N | N | G | 11200 |
| 303 | 315 | I | K | G | S | G | S | T | A | N | L | A | S | S | 12000 |
| 378 | 390 | E | T | T | Y | K | N | T | N | F | K | E | Y | L | 12500 |
| 411 | 423 | A | D | V | M | T | Y | I | H | S | M | N | S | T | 13200 |
| 180 | 192 | T | Q | L | C | L | I | G | C | K | P | P | I | G | 13500 |
| 399 | 411 | Q | F | I | F | Q | L | C | K | I | T | L | T | A | 13500 |
| 245 | 257 | E | V | P | L | D | I | C | T | S | I | C | K | Y | 14000 |
| 358 | 370 | T | V | V | D | T | T | R | S | T | N | M | S | L | 14500 |
| 155 | 167 | T | E | N | A | S | A | Y | A | A | N | A | G | V | 15500 |
| 365 | 377 | S | T | N | M | S | L | C | A | A | I | S | T | S | 16200 |
| 122 | 134 | Q | R | L | V | W | A | C | V | G | V | E | V | G | 16500 |
| 64 | 76 | G | T | S | R | L | L | A | V | G | H | P | Y | F | 17000 |
| 179 | 191 | Q | T | Q | L | C | L | I | G | C | K | P | P | I | 17000 |
| 427 | 439 | D | W | N | F | G | L | Q | P | P | P | G | G | T | 18000 |
| 396 | 408 | Y | D | L | Q | F | I | F | Q | L | C | K | I | T | 18200 |
| 84 | 96 | N | K | I | L | V | P | K | V | S | G | L | Q | Y | 19000 |
| 470 | 482 | Y | T | F | W | E | V | N | L | K | E | K | F | S | 19500 |
| 357 | 369 | V | T | V | V | D | T | T | R | S | T | N | M | S | 20000 |
| 65 | 77 | T | S | R | L | L | A | V | G | H | P | Y | F | P | 22200 |
| 290 | 302 | A | G | T | V | G | E | N | V | P | D | D | L | Y | 22200 |
| 373 | 385 | A | I | S | T | S | E | T | T | Y | K | N | T | N | 23500 |
| 323 | 335 | G | S | M | V | T | S | D | A | Q | I | F | N | K | 24000 |
| 279 | 291 | E | Q | M | F | V | R | H | L | F | N | R | A | G | 24500 |
| 85 | 97 | K | I | L | V | P | K | V | S | G | L | Q | Y | R | 26000 |
| 368 | 380 | M | S | L | C | A | A | I | S | T | S | E | T | T | 26500 |
| 108 | 120 | K | F | G | F | P | D | T | S | F | Y | N | P | D | 28000 |
| 34 | 46 | E | A | T | V | Y | L | P | P | V | P | V | S | K | 29200 |
| 248 | 260 | L | D | I | C | T | S | I | C | K | Y | P | D | Y | 30000 |
| 132 | 144 | E | V | G | R | G | Q | P | L | G | V | G | I | S | 31000 |
| 246 | 258 | V | P | L | D | I | C | T | S | I | C | K | Y | P | 31000 |
| 232 | 244 | G | A | M | D | F | T | T | L | Q | A | N | K | S | 33200 |
| 215 | 227 | E | L | I | N | T | V | I | Q | D | G | D | M | V | 34000 |
| 514 | 526 | T | P | T | T | S | S | T | S | T | T | A | K | R | 34500 |
| 230 | 242 | G | F | G | A | M | D | F | T | T | L | Q | A | N | 36000 |
| 340 | 352 | Q | R | A | Q | G | H | N | N | G | I | C | W | G | 37000 |
| 18 | 30 | V | N | V | H | H | I | F | F | Q | M | S | L | W | 39500 |
| 29 | 41 | L | W | L | P | S | E | A | T | V | Y | L | P | P | 40500 |
| 212 | 224 | P | P | L | E | L | I | N | T | V | I | Q | D | G | 40500 |
| 351 | 363 | W | G | N | Q | L | F | V | T | V | V | D | T | T | 40500 |
| 364 | 376 | R | S | T | N | M | S | L | C | A | A | I | S | T | 40500 |
| 178 | 190 | K | Q | T | Q | L | C | L | I | G | C | K | P | P | 42200 |
| 193 | 205 | E | H | W | G | K | G | S | P | C | T | N | V | A | 43000 |
| 443 | 455 | T | Y | R | F | V | T | Q | A | I | A | C | Q | K | 43000 |
| 307 | 319 | G | S | T | A | N | L | A | S | S | N | Y | F | P | 44000 |
| 515 | 527 | P | T | T | S | S | T | S | T | T | A | K | R | K | 45500 |
| 140 | 152 | G | V | G | I | S | G | H | P | L | L | N | K | L | 49500 |
| 379 | 391 | T | T | Y | K | N | T | N | F | K | E | Y | L | R | 53200 |
| 107 | 119 | N | K | F | G | F | P | D | T | S | F | Y | N | P | 53500 |
| 196 | 208 | G | K | G | S | P | C | T | N | V | A | V | N | P | 55200 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 469 | 481 | K | Y | T | F | W | E | V | N | L | K | E | K | F | 57500 |
| 55 | 67 | A | R | T | N | I | Y | Y | H | A | G | T | S | R | 58000 |
| 152 | 164 | L | D | D | T | E | N | A | S | A | Y | A | A | N | 58200 |
| 126 | 138 | W | A | C | V | G | V | E | V | G | R | G | Q | P | 59200 |
| 252 | 264 | T | S | I | C | K | Y | P | D | Y | L | K | M | V | 59500 |
| 171 | 183 | E | C | I | S | M | D | Y | K | Q | T | Q | L | C | 60500 |
| 63 | 75 | A | G | T | S | R | L | L | A | V | G | H | P | Y | 61200 |
| 217 | 229 | I | N | T | V | I | Q | D | G | D | M | V | H | T | 62000 |
| 147 | 159 | P | L | L | N | K | L | D | D | T | E | N | A | S | 62200 |
| 266 | 278 | E | P | Y | G | D | S | L | F | F | Y | L | R | R | 63500 |
| 224 | 236 | G | D | M | V | H | T | G | F | G | A | M | D | F | 64000 |
| 331 | 343 | Q | I | F | N | K | P | Y | W | L | Q | R | A | Q | 65000 |
| 284 | 296 | R | H | L | F | N | R | A | G | T | V | G | E | N | 66000 |
| 134 | 146 | G | R | G | Q | P | L | G | V | G | I | S | G | H | 66500 |
| 137 | 149 | Q | P | L | G | V | G | I | S | G | H | P | L | L | 67500 |
| 446 | 458 | F | V | T | Q | A | I | A | C | Q | K | H | T | P | 68200 |
| 504 | 516 | P | K | F | T | L | G | K | R | K | A | T | P | T | 69000 |
| 436 | 448 | P | G | G | T | L | E | D | T | Y | R | F | V | T | 71000 |
| 148 | 160 | L | L | N | K | L | D | D | T | E | N | A | S | A | 73000 |
| 42 | 54 | V | P | V | S | K | V | V | S | T | D | E | Y | V | 74200 |
| 36 | 48 | T | V | Y | L | P | P | V | P | V | S | K | V | V | 74500 |
| 176 | 188 | D | Y | K | Q | T | Q | L | C | L | I | G | C | K | 76500 |
| 384 | 396 | T | N | F | K | E | Y | L | R | H | G | E | E | Y | 78200 |
| 410 | 422 | T | A | D | V | M | T | Y | I | H | S | M | N | S | 83500 |
| 402 | 414 | F | Q | L | C | K | I | T | L | T | A | D | V | M | 91000 |
| 240 | 252 | Q | A | N | K | S | E | V | P | L | D | I | C | T | 93200 |
| 270 | 282 | D | S | L | F | F | Y | L | R | R | E | Q | M | F | 97500 |
| 131 | 143 | V | E | V | G | R | G | Q | P | L | G | V | G | I | 98000 |
| 360 | 372 | V | D | T | T | R | S | T | N | M | S | L | C | A | 98000 |
| 223 | 235 | D | G | D | M | V | H | T | G | F | G | A | M | D | 100000 |
| 494 | 506 | F | L | L | Q | A | G | L | K | A | K | P | K | F | 102000 |
| 89 | 101 | P | K | V | S | G | L | Q | Y | R | V | F | R | I | 105000 |
| 129 | 141 | V | G | V | E | V | G | R | G | Q | P | L | G | V | 105000 |
| 173 | 185 | I | S | M | D | Y | K | Q | T | Q | L | C | L | I | 112000 |
| 201 | 213 | C | T | N | V | A | V | N | P | G | D | C | P | P | 112000 |
| 313 | 325 | A | S | S | N | Y | F | P | T | P | S | G | S | M | 112000 |
| 213 | 225 | P | L | E | L | I | N | T | V | I | Q | D | G | D | 115000 |
| 306 | 318 | S | G | S | T | A | N | L | A | S | S | N | Y | F | 115000 |
| 332 | 344 | I | F | N | K | P | Y | W | L | Q | R | A | Q | G | 125000 |
| 56 | 68 | R | T | N | I | Y | Y | H | A | G | T | S | R | L | 130000 |
| 111 | 123 | F | P | D | T | S | F | Y | N | P | D | T | Q | R | 130000 |
| 367 | 379 | N | M | S | L | C | A | A | I | S | T | S | E | T | 132000 |
| 250 | 262 | I | C | T | S | I | C | K | Y | P | D | Y | L | K | 135000 |
| 11 | 23 | I | T | C | Y | E | N | D | V | N | V | H | H | I | 140000 |
| 79 | 91 | K | K | P | N | N | N | K | I | L | V | P | K | V | 140000 |
| 325 | 337 | M | V | T | S | D | A | Q | I | F | N | K | P | Y | 140000 |
| 475 | 487 | V | N | L | K | E | K | F | S | A | D | L | D | Q | 140000 |
| 127 | 139 | A | C | V | G | V | E | V | G | R | G | Q | P | L | 145000 |
| 19 | 31 | N | V | H | H | I | F | F | Q | M | S | L | W | L | 150000 |
| 54 | 66 | V | A | R | T | N | I | Y | Y | H | A | G | T | S | 150000 |
| 304 | 316 | K | G | S | G | S | T | A | N | L | A | S | S | N | 150000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 438 | 450 | G | T | L | E | D | T | Y | R | F | V | T | Q | A | 150000 |
| 471 | 483 | T | F | W | E | V | N | L | K | E | K | F | S | A | 150000 |
| 194 | 206 | H | W | G | K | G | S | P | C | T | N | V | A | V | 160000 |
| 345 | 357 | H | N | N | G | I | C | W | G | N | Q | L | F | V | 160000 |
| 247 | 259 | P | L | D | I | C | T | S | I | C | K | Y | P | D | 170000 |
| 489 | 501 | P | L | G | R | K | F | L | L | Q | A | G | L | K | 170000 |
| 321 | 333 | P | S | G | S | M | V | T | S | D | A | Q | I | F | 172000 |
| 493 | 505 | K | F | L | L | Q | A | G | L | K | A | K | P | K | 175000 |
| 283 | 295 | V | R | H | L | F | N | R | A | G | T | V | G | E | 180000 |
| 27 | 39 | M | S | L | W | L | P | S | E | A | T | V | Y | L | 190000 |
| 7 | 19 | Y | I | L | V | I | T | C | Y | E | N | D | V | N | 192000 |
| 200 | 212 | P | C | T | N | V | A | V | N | P | G | D | C | P | 195000 |
| 356 | 368 | F | V | T | V | V | D | T | T | R | S | T | N | M | 195000 |
| 136 | 148 | G | Q | P | L | G | V | G | I | S | G | H | P | L | 200000 |
| 198 | 210 | G | S | P | C | T | N | V | A | V | N | P | G | D | 200000 |
| 338 | 350 | W | L | Q | R | A | Q | G | H | N | N | G | I | C | 205000 |
| 363 | 375 | T | R | S | T | N | M | S | L | C | A | A | I | S | 205000 |
| 397 | 409 | D | L | Q | F | I | F | Q | L | C | K | I | T | L | 220000 |
| 490 | 502 | L | G | R | K | F | L | L | Q | A | G | L | K | A | 225000 |
| 114 | 126 | T | S | F | Y | N | P | D | T | Q | R | L | V | W | 230000 |
| 317 | 329 | Y | F | P | T | P | S | G | S | M | V | T | S | D | 230000 |
| 191 | 203 | I | G | E | H | W | G | K | G | S | P | C | T | N | 235000 |
| 352 | 364 | G | N | Q | L | F | V | T | V | V | D | T | T | R | 235000 |
| 465 | 477 | D | P | L | K | K | Y | T | F | W | E | V | N | L | 250000 |
| 13 | 25 | C | Y | E | N | D | V | N | V | H | H | I | F | F | 252000 |
| 312 | 324 | L | A | S | S | N | Y | F | P | T | P | S | G | S | 255000 |
| 272 | 284 | L | F | F | Y | L | R | R | E | Q | M | F | V | R | 260000 |
| 156 | 168 | E | N | A | S | A | Y | A | A | N | A | G | V | D | 262000 |
| 305 | 317 | G | S | G | S | T | A | N | L | A | S | S | N | Y | 262000 |
| 349 | 361 | I | C | W | G | N | Q | L | F | V | T | V | V | D | 265000 |
| 516 | 528 | T | T | S | S | T | S | T | T | A | K | R | K | K | 272000 |
| 473 | 485 | W | E | V | N | L | K | E | K | F | S | A | D | L | 275000 |
| 47 | 59 | V | V | S | T | D | E | Y | V | A | R | T | N | I | 282000 |
| 348 | 360 | G | I | C | W | G | N | Q | L | F | V | T | V | V | 282000 |
| 406 | 418 | K | I | T | L | T | A | D | V | M | T | Y | I | H | 290000 |
| 437 | 449 | G | G | T | L | E | D | T | Y | R | F | V | T | Q | 290000 |
| 413 | 425 | V | M | T | Y | I | H | S | M | N | S | T | I | L | 305000 |
| 255 | 267 | C | K | Y | P | D | Y | L | K | M | V | S | E | P | 315000 |
| 62 | 74 | H | A | G | T | S | R | L | L | A | V | G | H | P | 330000 |
| 320 | 332 | T | P | S | G | S | M | V | T | S | D | A | Q | I | 330000 |
| 506 | 518 | F | T | L | G | K | R | K | A | T | P | T | T | S | 340000 |
| 298 | 310 | P | D | D | L | Y | I | K | G | S | G | S | T | A | 342000 |
| 401 | 413 | I | F | Q | L | C | K | I | T | L | T | A | D | V | 352000 |
| 119 | 131 | P | D | T | Q | R | L | V | W | A | C | V | G | V | 355000 |
| 299 | 311 | D | D | L | Y | I | K | G | S | G | S | T | A | N | 360000 |
| 286 | 298 | L | F | N | R | A | G | T | V | G | E | N | V | P | 365000 |
| 99 | 111 | F | R | I | H | L | P | D | P | N | K | F | G | F | 380000 |
| 268 | 280 | Y | G | D | S | L | F | F | Y | L | R | R | E | Q | 380000 |
| 308 | 320 | S | T | A | N | L | A | S | S | N | Y | F | P | T | 380000 |
| 153 | 165 | D | D | T | E | N | A | S | A | Y | A | A | N | A | 382000 |
| 330 | 342 | A | Q | I | F | N | K | P | Y | W | L | Q | R | A | 390000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 243 | 255 | K | S | E | V | P | L | D | I | C | T | S | I | C | 392000 |
| 350 | 362 | C | W | G | N | Q | L | F | V | T | V | V | D | T | 395000 |
| 222 | 234 | Q | D | G | D | M | V | H | T | G | F | G | A | M | 400000 |
| 508 | 520 | L | G | K | R | K | A | T | P | T | T | S | S | T | 415000 |
| 262 | 274 | K | M | V | S | E | P | Y | G | D | S | L | F | F | 430000 |
| 416 | 428 | Y | I | H | S | M | N | S | T | I | L | E | D | W | 440000 |
| 404 | 416 | L | C | K | I | T | L | T | A | D | V | M | T | Y | 450000 |
| 189 | 201 | P | P | I | G | E | H | W | G | K | G | S | P | C | 455000 |
| 92 | 104 | S | G | L | Q | Y | R | V | F | R | I | H | L | P | 475000 |
| 226 | 238 | M | V | H | T | G | F | G | A | M | D | F | T | T | 480000 |
| 32 | 44 | P | S | E | A | T | V | Y | L | P | P | V | P | V | 490000 |
| 109 | 121 | F | G | F | P | D | T | S | F | Y | N | P | D | T | 492000 |
| 441 | 453 | E | D | T | Y | R | F | V | T | Q | A | I | A | C | 492000 |
| 488 | 500 | F | P | L | G | R | K | F | L | L | Q | A | G | L | 510000 |
| 403 | 415 | Q | L | C | K | I | T | L | T | A | D | V | M | T | 515000 |
| 467 | 479 | L | K | K | Y | T | F | W | E | V | N | L | K | E | 515000 |
| 482 | 494 | S | A | D | L | D | Q | F | P | L | G | R | K | F | 525000 |
| 177 | 189 | Y | K | Q | T | Q | L | C | L | I | G | C | K | P | 560000 |
| 511 | 523 | R | K | A | T | P | T | T | S | S | T | S | T | T | 560000 |
| 50 | 62 | T | D | E | Y | V | A | R | T | N | I | Y | Y | H | 572000 |
| 10 | 22 | V | I | T | C | Y | E | N | D | V | N | V | H | H | 575000 |
| 509 | 521 | G | K | R | K | A | T | P | T | T | S | S | T | S | 610000 |
| 167 | 179 | V | D | N | R | E | C | I | S | M | D | Y | K | Q | 630000 |
| 294 | 306 | G | E | N | V | P | D | D | L | Y | I | K | G | S | 642000 |
| 149 | 161 | L | N | K | L | D | D | T | E | N | A | S | A | Y | 650000 |
| 409 | 421 | L | T | A | D | V | M | T | Y | I | H | S | M | N | 650000 |
| 477 | 489 | L | K | E | K | F | S | A | D | L | D | Q | F | P | 665000 |
| 76 | 88 | F | P | I | K | K | P | N | N | N | K | I | L | V | 700000 |
| 486 | 498 | D | Q | F | P | L | G | R | K | F | L | L | Q | A | 710000 |
| 82 | 94 | N | N | N | K | I | L | V | P | K | V | S | G | L | 720000 |
| 451 | 463 | I | A | C | Q | K | H | T | P | P | A | P | K | E | 722000 |
| 112 | 124 | P | D | T | S | F | Y | N | P | D | T | Q | R | L | 730000 |
| 157 | 169 | N | A | S | A | Y | A | A | N | A | G | V | D | N | 730000 |
| 311 | 323 | N | L | A | S | S | N | Y | F | P | T | P | S | G | 730000 |
| 70 | 82 | A | V | G | H | P | Y | F | P | I | K | K | P | N | 735000 |
| 91 | 103 | V | S | G | L | Q | Y | R | V | F | R | I | H | L | 745000 |
| 220 | 232 | V | I | Q | D | G | D | M | V | H | T | G | F | G | 765000 |
| 265 | 277 | S | E | P | Y | G | D | S | L | F | F | Y | L | R | 765000 |
| 30 | 42 | W | L | P | S | E | A | T | V | Y | L | P | P | V | 785000 |
| 20 | 32 | V | H | H | I | F | F | Q | M | S | L | W | L | P | 795000 |
| 154 | 166 | D | T | E | N | A | S | A | Y | A | A | N | A | G | 795000 |
| 369 | 381 | S | L | C | A | A | I | S | T | S | E | T | T | Y | 840000 |
| 383 | 395 | N | T | N | F | K | E | Y | L | R | H | G | E | E | 882000 |
| 480 | 492 | K | F | S | A | D | L | D | Q | F | P | L | G | R | 895000 |
| 343 | 355 | Q | G | H | N | N | G | I | C | W | G | N | Q | L | 900000 |
| 113 | 125 | D | T | S | F | Y | N | P | D | T | Q | R | L | V | 920000 |
| 121 | 133 | T | Q | R | L | V | W | A | C | V | G | V | E | V | 922000 |
| 341 | 353 | R | A | Q | G | H | N | N | G | I | C | W | G | N | 925000 |
| 174 | 186 | S | M | D | Y | K | Q | T | Q | L | C | L | I | G | 945000 |
| 491 | 503 | G | R | K | F | L | L | Q | A | G | L | K | A | K | 960000 |
| 17 | 29 | D | V | N | V | H | H | I | F | F | Q | M | S | L | 985000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 33 | 45 | S | E | A | T | V | Y | L | P | P | V | P | V | S | 985000 |
| 165 | 177 | A | G | V | D | N | R | E | C | I | S | M | D | Y | 985000 |
| 14 | 26 | Y | E | N | D | V | N | V | H | H | I | F | F | Q | 1000000 |
| 15 | 27 | E | N | D | V | N | V | H | H | I | F | F | Q | M | 1000000 |
| 24 | 36 | F | F | Q | M | S | L | W | L | P | S | E | A | T | 1000000 |
| 26 | 38 | Q | M | S | L | W | L | P | S | E | A | T | V | Y | 1000000 |
| 31 | 43 | L | P | S | E | A | T | V | Y | L | P | P | V | P | 1000000 |
| 37 | 49 | V | Y | L | P | P | V | P | V | S | K | V | V | S | 1000000 |
| 38 | 50 | Y | L | P | P | V | P | V | S | K | V | V | S | T | 1000000 |
| 39 | 51 | L | P | P | V | P | V | S | K | V | V | S | T | D | 1000000 |
| 40 | 52 | P | P | V | P | V | S | K | V | V | S | T | D | E | 1000000 |
| 41 | 53 | P | V | P | V | S | K | V | V | S | T | D | E | Y | 1000000 |
| 43 | 55 | P | V | S | K | V | V | S | T | D | E | Y | V | A | 1000000 |
| 44 | 56 | V | S | K | V | V | S | T | D | E | Y | V | A | R | 1000000 |
| 48 | 60 | V | S | T | D | E | Y | V | A | R | T | N | I | Y | 1000000 |
| 49 | 61 | S | T | D | E | Y | V | A | R | T | N | I | Y | Y | 1000000 |
| 60 | 72 | Y | Y | H | A | G | T | S | R | L | L | A | V | G | 1000000 |
| 61 | 73 | Y | H | A | G | T | S | R | L | L | A | V | G | H | 1000000 |
| 68 | 80 | L | L | A | V | G | H | P | Y | F | P | I | K | K | 1000000 |
| 69 | 81 | L | A | V | G | H | P | Y | F | P | I | K | K | P | 1000000 |
| 71 | 83 | V | G | H | P | Y | F | P | I | K | K | P | N | N | 1000000 |
| 72 | 84 | G | H | P | Y | F | P | I | K | K | P | N | N | N | 1000000 |
| 74 | 86 | P | Y | F | P | I | K | K | P | N | N | N | K | I | 1000000 |
| 75 | 87 | Y | F | P | I | K | K | P | N | N | N | K | I | L | 1000000 |
| 77 | 89 | P | I | K | K | P | N | N | N | K | I | L | V | P | 1000000 |
| 78 | 90 | I | K | K | P | N | N | N | K | I | L | V | P | K | 1000000 |
| 80 | 92 | K | P | N | N | N | K | I | L | V | P | K | V | S | 1000000 |
| 81 | 93 | P | N | N | N | K | I | L | V | P | K | V | S | G | 1000000 |
| 83 | 95 | N | N | K | I | L | V | P | K | V | S | G | L | Q | 1000000 |
| 86 | 98 | I | L | V | P | K | V | S | G | L | Q | Y | R | V | 1000000 |
| 87 | 99 | L | V | P | K | V | S | G | L | Q | Y | R | V | F | 1000000 |
| 88 | 100 | V | P | K | V | S | G | L | Q | Y | R | V | F | R | 1000000 |
| 90 | 102 | K | V | S | G | L | Q | Y | R | V | F | R | I | H | 1000000 |
| 93 | 105 | G | L | Q | Y | R | V | F | R | I | H | L | P | D | 1000000 |
| 95 | 107 | Q | Y | R | V | F | R | I | H | L | P | D | P | N | 1000000 |
| 98 | 110 | V | F | R | I | H | L | P | D | P | N | K | F | G | 1000000 |
| 100 | 112 | R | I | H | L | P | D | P | N | K | F | G | F | P | 1000000 |
| 101 | 113 | I | H | L | P | D | P | N | K | F | G | F | P | D | 1000000 |
| 102 | 114 | H | L | P | D | P | N | K | F | G | F | P | D | T | 1000000 |
| 103 | 115 | L | P | D | P | N | K | F | G | F | P | D | T | S | 1000000 |
| 104 | 116 | P | D | P | N | K | F | G | F | P | D | T | S | F | 1000000 |
| 105 | 117 | D | P | N | K | F | G | F | P | D | T | S | F | Y | 1000000 |
| 106 | 118 | P | N | K | F | G | F | P | D | T | S | F | Y | N | 1000000 |
| 110 | 122 | G | F | P | D | T | S | F | Y | N | P | D | T | Q | 1000000 |
| 116 | 128 | F | Y | N | P | D | T | Q | R | L | V | W | A | C | 1000000 |
| 117 | 129 | Y | N | P | D | T | Q | R | L | V | W | A | C | V | 1000000 |
| 118 | 130 | N | P | D | T | Q | R | L | V | W | A | C | V | G | 1000000 |
| 125 | 137 | V | W | A | C | V | G | V | E | V | G | R | G | Q | 1000000 |
| 128 | 140 | C | V | G | V | E | V | G | R | G | Q | P | L | G | 1000000 |
| 130 | 142 | G | V | E | V | G | R | G | Q | P | L | G | V | G | 1000000 |
| 133 | 145 | V | G | R | G | Q | P | L | G | V | G | I | S | G | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 135 | 147 | R | G | Q | P | L | G | V | G | I | S | G | H | P | 1000000 |
| 142 | 154 | G | I | S | G | H | P | L | L | N | K | L | D | D | 1000000 |
| 143 | 155 | I | S | G | H | P | L | L | N | K | L | D | D | T | 1000000 |
| 144 | 156 | S | G | H | P | L | L | N | K | L | D | D | T | E | 1000000 |
| 145 | 157 | G | H | P | L | L | N | K | L | D | D | T | E | N | 1000000 |
| 146 | 158 | H | P | L | L | N | K | L | D | D | T | E | N | A | 1000000 |
| 151 | 163 | K | L | D | D | T | E | N | A | S | A | Y | A | A | 1000000 |
| 158 | 170 | A | S | A | Y | A | A | N | A | G | V | D | N | R | 1000000 |
| 160 | 172 | A | Y | A | A | N | A | G | V | D | N | R | E | C | 1000000 |
| 161 | 173 | Y | A | A | N | A | G | V | D | N | R | E | C | I | 1000000 |
| 162 | 174 | A | A | N | A | G | V | D | N | R | E | C | I | S | 1000000 |
| 163 | 175 | A | N | A | G | V | D | N | R | E | C | I | S | M | 1000000 |
| 166 | 178 | G | V | D | N | R | E | C | I | S | M | D | Y | K | 1000000 |
| 168 | 180 | D | N | R | E | C | I | S | M | D | Y | K | Q | T | 1000000 |
| 169 | 181 | N | R | E | C | I | S | M | D | Y | K | Q | T | Q | 1000000 |
| 170 | 182 | R | E | C | I | S | M | D | Y | K | Q | T | Q | L | 1000000 |
| 172 | 184 | C | I | S | M | D | Y | K | Q | T | Q | L | C | L | 1000000 |
| 181 | 193 | Q | L | C | L | I | G | C | K | P | P | I | G | E | 1000000 |
| 183 | 195 | C | L | I | G | C | K | P | P | I | G | E | H | W | 1000000 |
| 184 | 196 | L | I | G | C | K | P | P | I | G | E | H | W | G | 1000000 |
| 185 | 197 | I | G | C | K | P | P | I | G | E | H | W | G | K | 1000000 |
| 186 | 198 | G | C | K | P | P | I | G | E | H | W | G | K | G | 1000000 |
| 187 | 199 | C | K | P | P | I | G | E | H | W | G | K | G | S | 1000000 |
| 188 | 200 | K | P | P | I | G | E | H | W | G | K | G | S | P | 1000000 |
| 190 | 202 | P | I | G | E | H | W | G | K | G | S | P | C | T | 1000000 |
| 192 | 204 | G | E | H | W | G | K | G | S | P | C | T | N | V | 1000000 |
| 195 | 207 | W | G | K | G | S | P | C | T | N | V | A | V | N | 1000000 |
| 197 | 209 | K | G | S | P | C | T | N | V | A | V | N | P | G | 1000000 |
| 199 | 211 | S | P | C | T | N | V | A | V | N | P | G | D | C | 1000000 |
| 203 | 215 | N | V | A | V | N | P | G | D | C | P | P | L | E | 1000000 |
| 205 | 217 | A | V | N | P | G | D | C | P | P | L | E | L | I | 1000000 |
| 206 | 218 | V | N | P | G | D | C | P | P | L | E | L | I | N | 1000000 |
| 207 | 219 | N | P | G | D | C | P | P | L | E | L | I | N | T | 1000000 |
| 208 | 220 | P | G | D | C | P | P | L | E | L | I | N | T | V | 1000000 |
| 209 | 221 | G | D | C | P | P | L | E | L | I | N | T | V | I | 1000000 |
| 210 | 222 | D | C | P | P | L | E | L | I | N | T | V | I | Q | 1000000 |
| 211 | 223 | C | P | P | L | E | L | I | N | T | V | I | Q | D | 1000000 |
| 216 | 228 | L | I | N | T | V | I | Q | D | G | D | M | V | H | 1000000 |
| 221 | 233 | I | Q | D | G | D | M | V | H | T | G | F | G | A | 1000000 |
| 227 | 239 | V | H | T | G | F | G | A | M | D | F | T | T | L | 1000000 |
| 228 | 240 | H | T | G | F | G | A | M | D | F | T | T | L | Q | 1000000 |
| 233 | 245 | A | M | D | F | T | T | L | Q | A | N | K | S | E | 1000000 |
| 235 | 247 | D | F | T | T | L | Q | A | N | K | S | E | V | P | 1000000 |
| 236 | 248 | F | T | T | L | Q | A | N | K | S | E | V | P | L | 1000000 |
| 238 | 250 | T | L | Q | A | N | K | S | E | V | P | L | D | I | 1000000 |
| 239 | 251 | L | Q | A | N | K | S | E | V | P | L | D | I | C | 1000000 |
| 241 | 253 | A | N | K | S | E | V | P | L | D | I | C | T | S | 1000000 |
| 242 | 254 | N | K | S | E | V | P | L | D | I | C | T | S | I | 1000000 |
| 244 | 256 | S | E | V | P | L | D | I | C | T | S | I | C | K | 1000000 |
| 249 | 261 | D | I | C | T | S | I | C | K | Y | P | D | Y | L | 1000000 |
| 251 | 263 | C | T | S | I | C | K | Y | P | D | Y | L | K | M | 1000000 |

| | | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--|---------|
| 253 | 265 | S | I | C | K | Y | P | D | Y | L | K | M | V | S | | 1000000 |
| 254 | 266 | I | C | K | Y | P | D | Y | L | K | M | V | S | E | | 1000000 |
| 256 | 268 | K | Y | P | D | Y | L | K | M | V | S | E | P | Y | | 1000000 |
| 257 | 269 | Y | P | D | Y | L | K | M | V | S | E | P | Y | G | | 1000000 |
| 260 | 272 | Y | L | K | M | V | S | E | P | Y | G | D | S | L | | 1000000 |
| 263 | 275 | M | V | S | E | P | Y | G | D | S | L | F | F | Y | | 1000000 |
| 264 | 276 | V | S | E | P | Y | G | D | S | L | F | F | Y | L | | 1000000 |
| 267 | 279 | P | Y | G | D | S | L | F | F | Y | L | R | R | E | | 1000000 |
| 269 | 281 | G | D | S | L | F | F | Y | L | R | R | E | Q | M | | 1000000 |
| 275 | 287 | Y | L | R | R | E | Q | M | F | V | R | H | L | F | | 1000000 |
| 276 | 288 | L | R | R | E | Q | M | F | V | R | H | L | F | N | | 1000000 |
| 277 | 289 | R | R | E | Q | M | F | V | R | H | L | F | N | R | | 1000000 |
| 278 | 290 | R | E | Q | M | F | V | R | H | L | F | N | R | A | | 1000000 |
| 282 | 294 | F | V | R | H | L | F | N | R | A | G | T | V | G | | 1000000 |
| 287 | 299 | F | N | R | A | G | T | V | G | E | N | V | P | D | | 1000000 |
| 288 | 300 | N | R | A | G | T | V | G | E | N | V | P | D | D | | 1000000 |
| 289 | 301 | R | A | G | T | V | G | E | N | V | P | D | D | L | | 1000000 |
| 292 | 304 | T | V | G | E | N | V | P | D | D | L | Y | I | K | | 1000000 |
| 293 | 305 | V | G | E | N | V | P | D | D | L | Y | I | K | G | | 1000000 |
| 295 | 307 | E | N | V | P | D | D | L | Y | I | K | G | S | G | | 1000000 |
| 296 | 308 | N | V | P | D | D | L | Y | I | K | G | S | G | S | | 1000000 |
| 297 | 309 | V | P | D | D | L | Y | I | K | G | S | G | S | T | | 1000000 |
| 302 | 314 | Y | I | K | G | S | G | S | T | A | N | L | A | S | | 1000000 |
| 314 | 326 | S | S | N | Y | F | P | T | P | S | G | S | M | V | | 1000000 |
| 316 | 328 | N | Y | F | P | T | P | S | G | S | M | V | T | S | | 1000000 |
| 318 | 330 | F | P | T | P | S | G | S | M | V | T | S | D | A | | 1000000 |
| 319 | 331 | P | T | P | S | G | S | M | V | T | S | D | A | Q | | 1000000 |
| 322 | 334 | S | G | S | M | V | T | S | D | A | Q | I | F | N | | 1000000 |
| 326 | 338 | V | T | S | D | A | Q | I | F | N | K | P | Y | W | | 1000000 |
| 327 | 339 | T | S | D | A | Q | I | F | N | K | P | Y | W | L | | 1000000 |
| 328 | 340 | S | D | A | Q | I | F | N | K | P | Y | W | L | Q | | 1000000 |
| 333 | 345 | F | N | K | P | Y | W | L | Q | R | A | Q | G | H | | 1000000 |
| 334 | 346 | N | K | P | Y | W | L | Q | R | A | Q | G | H | N | | 1000000 |
| 339 | 351 | L | Q | R | A | Q | G | H | N | N | G | I | C | W | | 1000000 |
| 344 | 356 | G | H | N | N | G | I | C | W | G | N | Q | L | F | | 1000000 |
| 346 | 358 | N | N | G | I | C | W | G | N | Q | L | F | V | T | | 1000000 |
| 359 | 371 | V | V | D | T | T | R | S | T | N | M | S | L | C | | 1000000 |
| 370 | 382 | L | C | A | A | I | S | T | S | E | T | T | Y | K | | 1000000 |
| 371 | 383 | C | A | A | I | S | T | S | E | T | T | Y | K | N | | 1000000 |
| 374 | 386 | I | S | T | S | E | T | T | Y | K | N | T | N | F | | 1000000 |
| 375 | 387 | S | T | S | E | T | T | Y | K | N | T | N | F | K | | 1000000 |
| 376 | 388 | T | S | E | T | T | Y | K | N | T | N | F | K | E | | 1000000 |
| 377 | 389 | S | E | T | T | Y | K | N | T | N | F | K | E | Y | | 1000000 |
| 380 | 392 | T | Y | K | N | T | N | F | K | E | Y | L | R | H | | 1000000 |
| 381 | 393 | Y | K | N | T | N | F | K | E | Y | L | R | H | G | | 1000000 |
| 382 | 394 | K | N | T | N | F | K | E | Y | L | R | H | G | E | | 1000000 |
| 385 | 397 | N | F | K | E | Y | L | R | H | G | E | E | Y | D | | 1000000 |
| 386 | 398 | F | K | E | Y | L | R | H | G | E | E | Y | D | L | | 1000000 |
| 389 | 401 | Y | L | R | H | G | E | E | Y | D | L | Q | F | I | | 1000000 |
| 390 | 402 | L | R | H | G | E | E | Y | D | L | Q | F | I | F | | 1000000 |
| 391 | 403 | R | H | G | E | E | Y | D | L | Q | F | I | F | Q | | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 392 | 404 | H | G | E | E | Y | D | L | Q | F | I | F | Q | L | 1000000 |
| 393 | 405 | G | E | E | Y | D | L | Q | F | I | F | Q | L | C | 1000000 |
| 395 | 407 | E | Y | D | L | Q | F | I | F | Q | L | C | K | I | 1000000 |
| 417 | 429 | I | H | S | M | N | S | T | I | L | E | D | W | N | 1000000 |
| 419 | 431 | S | M | N | S | T | I | L | E | D | W | N | F | G | 1000000 |
| 420 | 432 | M | N | S | T | I | L | E | D | W | N | F | G | L | 1000000 |
| 423 | 435 | T | I | L | E | D | W | N | F | G | L | Q | P | P | 1000000 |
| 424 | 436 | I | L | E | D | W | N | F | G | L | Q | P | P | P | 1000000 |
| 425 | 437 | L | E | D | W | N | F | G | L | Q | P | P | P | G | 1000000 |
| 429 | 441 | N | F | G | L | Q | P | P | P | G | G | T | L | E | 1000000 |
| 430 | 442 | F | G | L | Q | P | P | P | G | G | T | L | E | D | 1000000 |
| 431 | 443 | G | L | Q | P | P | P | G | G | T | L | E | D | T | 1000000 |
| 432 | 444 | L | Q | P | P | P | G | G | T | L | E | D | T | Y | 1000000 |
| 433 | 445 | Q | P | P | P | G | G | T | L | E | D | T | Y | R | 1000000 |
| 434 | 446 | P | P | P | G | G | T | L | E | D | T | Y | R | F | 1000000 |
| 435 | 447 | P | P | G | G | T | L | E | D | T | Y | R | F | V | 1000000 |
| 439 | 451 | T | L | E | D | T | Y | R | F | V | T | Q | A | I | 1000000 |
| 440 | 452 | L | E | D | T | Y | R | F | V | T | Q | A | I | A | 1000000 |
| 447 | 459 | V | T | Q | A | I | A | C | Q | K | H | T | P | P | 1000000 |
| 448 | 460 | T | Q | A | I | A | C | Q | K | H | T | P | P | A | 1000000 |
| 450 | 462 | A | I | A | C | Q | K | H | T | P | P | A | P | K | 1000000 |
| 452 | 464 | A | C | Q | K | H | T | P | P | A | P | K | E | D | 1000000 |
| 453 | 465 | C | Q | K | H | T | P | P | A | P | K | E | D | D | 1000000 |
| 454 | 466 | Q | K | H | T | P | P | A | P | K | E | D | D | P | 1000000 |
| 455 | 467 | K | H | T | P | P | A | P | K | E | D | D | P | L | 1000000 |
| 456 | 468 | H | T | P | P | A | P | K | E | D | D | P | L | K | 1000000 |
| 457 | 469 | T | P | P | A | P | K | E | D | D | P | L | K | K | 1000000 |
| 458 | 470 | P | P | A | P | K | E | D | D | P | L | K | K | Y | 1000000 |
| 459 | 471 | P | A | P | K | E | D | D | P | L | K | K | Y | T | 1000000 |
| 460 | 472 | A | P | K | E | D | D | P | L | K | K | Y | T | F | 1000000 |
| 461 | 473 | P | K | E | D | D | P | L | K | K | Y | T | F | W | 1000000 |
| 462 | 474 | K | E | D | D | P | L | K | K | Y | T | F | W | E | 1000000 |
| 463 | 475 | E | D | D | P | L | K | K | Y | T | F | W | E | V | 1000000 |
| 464 | 476 | D | D | P | L | K | K | Y | T | F | W | E | V | N | 1000000 |
| 466 | 478 | P | L | K | K | Y | T | F | W | E | V | N | L | K | 1000000 |
| 472 | 484 | F | W | E | V | N | L | K | E | K | F | S | A | D | 1000000 |
| 474 | 486 | E | V | N | L | K | E | K | F | S | A | D | L | D | 1000000 |
| 476 | 488 | N | L | K | E | K | F | S | A | D | L | D | Q | F | 1000000 |
| 478 | 490 | K | E | K | F | S | A | D | L | D | Q | F | P | L | 1000000 |
| 481 | 493 | F | S | A | D | L | D | Q | F | P | L | G | R | K | 1000000 |
| 483 | 495 | A | D | L | D | Q | F | P | L | G | R | K | F | L | 1000000 |
| 484 | 496 | D | L | D | Q | F | P | L | G | R | K | F | L | L | 1000000 |
| 485 | 497 | L | D | Q | F | P | L | G | R | K | F | L | L | Q | 1000000 |
| 487 | 499 | Q | F | P | L | G | R | K | F | L | L | Q | A | G | 1000000 |
| 495 | 507 | L | L | Q | A | G | L | K | A | K | P | K | F | T | 1000000 |
| 496 | 508 | L | Q | A | G | L | K | A | K | P | K | F | T | L | 1000000 |
| 497 | 509 | Q | A | G | L | K | A | K | P | K | F | T | L | G | 1000000 |
| 498 | 510 | A | G | L | K | A | K | P | K | F | T | L | G | K | 1000000 |
| 499 | 511 | G | L | K | A | K | P | K | F | T | L | G | K | R | 1000000 |
| 500 | 512 | L | K | A | K | P | K | F | T | L | G | K | R | K | 1000000 |
| 501 | 513 | K | A | K | P | K | F | T | L | G | K | R | K | A | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 502 | 514 | A | K | P | K | F | T | L | G | K | R | K | A | T | 1000000 |
| 503 | 515 | K | P | K | F | T | L | G | K | R | K | A | T | P | 1000000 |
| 505 | 517 | K | F | T | L | G | K | R | K | A | T | P | T | T | 1000000 |
| 507 | 519 | T | L | G | K | R | K | A | T | P | T | T | S | S | 1000000 |
| 510 | 522 | K | R | K | A | T | P | T | T | S | S | T | S | T | 1000000 |
| 512 | 524 | K | A | T | P | T | T | S | S | T | S | T | T | A | 1000000 |
| 513 | 525 | A | T | P | T | T | S | S | T | S | T | T | A | K | 1000000 |
| 517 | 529 | T | S | S | T | S | T | T | A | K | R | K | K | R | 1000000 |
| 518 | 530 | S | S | T | S | T | T | A | K | R | K | K | R | K | 1000000 |
| 519 | 531 | S | T | S | T | T | A | K | R | K | K | R | K | L | 1000000 |

| | | | | | | | | | | | | | | | IC50 DRB1*0101(nM) |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------------------|
| 240 | 252 | P | A | F | V | T | T | P | T | K | L | I | T | Y | 2.7 |
| 444 | 456 | G | D | F | Y | L | H | P | S | Y | Y | M | L | R | 3.35 |
| 52 | 64 | G | V | F | F | G | G | L | G | I | G | T | G | S | 7.2 |
| 301 | 313 | I | R | Y | S | R | I | G | N | K | Q | T | L | R | 11.5 |
| 200 | 212 | T | F | I | V | S | T | N | P | N | T | V | T | S | 13 |
| 445 | 457 | D | F | Y | L | H | P | S | Y | Y | M | L | R | K | 13 |
| 70 | 82 | T | G | Y | I | P | L | G | T | R | P | P | T | A | 14 |
| 418 | 430 | I | N | I | T | D | Q | A | P | S | L | I | P | I | 19 |
| 161 | 173 | P | T | F | T | D | P | S | V | L | Q | P | P | T | 32 |
| 144 | 156 | A | I | L | D | I | N | N | T | V | T | T | V | T | 34 |
| 150 | 162 | N | T | V | T | T | V | T | T | H | N | N | P | T | 40 |
| 285 | 297 | D | I | V | A | L | H | R | P | A | L | T | S | R | 40 |
| 199 | 211 | D | T | F | I | V | S | T | N | P | N | T | V | T | 44 |
| 196 | 208 | I | P | M | D | T | F | I | V | S | T | N | P | N | 61.5 |
| 208 | 220 | N | T | V | T | S | S | T | P | I | P | G | S | R | 63 |
| 17 | 29 | Q | L | Y | K | T | C | K | Q | A | G | T | C | P | 68 |
| 388 | 400 | T | S | L | S | G | Y | I | P | A | N | T | T | I | 69 |
| 112 | 124 | T | S | F | I | D | A | G | A | P | T | S | V | P | 70.5 |
| 347 | 359 | S | T | Y | T | T | T | S | H | A | A | S | P | T | 79.5 |
| 450 | 462 | P | S | Y | Y | M | L | R | K | R | R | K | R | L | 102 |
| 43 | 55 | E | Q | I | L | Q | Y | G | S | M | G | V | F | F | 135 |
| 325 | 337 | H | Y | Y | Y | D | L | S | T | I | D | P | A | E | 135 |
| 281 | 293 | P | D | F | L | D | I | V | A | L | H | R | P | A | 140 |
| 265 | 277 | T | L | Y | F | S | S | N | D | N | S | I | N | I | 142 |
| 266 | 278 | L | Y | F | S | S | N | D | N | S | I | N | I | A | 160 |
| 428 | 440 | I | P | I | V | P | G | S | P | Q | Y | T | I | I | 190 |
| 292 | 304 | P | A | L | T | S | R | R | T | G | I | R | Y | S | 210 |
| 425 | 437 | P | S | L | I | P | I | V | P | G | S | P | Q | Y | 222 |
| 282 | 294 | D | F | L | D | I | V | A | L | H | R | P | A | L | 275 |
| 84 | 96 | D | T | L | A | P | V | R | P | P | L | T | V | D | 305 |
| 404 | 416 | G | A | Y | N | I | P | L | V | S | G | P | D | I | 432 |
| 255 | 267 | P | A | Y | E | G | I | D | V | D | N | T | L | Y | 462 |
| 51 | 63 | M | G | V | F | F | G | G | L | G | I | G | T | G | 550 |
| 310 | 322 | Q | T | L | R | T | R | S | G | K | S | I | G | A | 600 |
| 241 | 253 | A | F | V | T | T | P | T | K | L | I | T | Y | D | 650 |
| 408 | 420 | I | P | L | V | S | G | P | D | I | P | I | N | I | 725 |
| 233 | 245 | Q | Q | V | K | V | V | D | P | A | F | V | T | T | 955 |
| 181 | 193 | F | T | L | S | S | S | T | I | S | T | H | N | Y | 1100 |
| 400 | 412 | I | P | F | G | G | A | Y | N | I | P | L | V | S | 1100 |
| 49 | 61 | G | S | M | G | V | F | F | G | G | L | G | I | G | 1150 |
| 398 | 410 | T | T | I | P | F | G | G | A | Y | N | I | P | L | 1400 |
| 103 | 115 | P | S | I | V | S | L | V | E | E | T | S | F | I | 1700 |
| 235 | 247 | V | K | V | V | D | P | A | F | V | T | T | P | T | 1900 |
| 226 | 238 | G | L | Y | S | R | T | T | Q | Q | V | K | V | V | 2050 |
| 167 | 179 | S | V | L | Q | P | P | T | P | A | E | T | G | G | 2250 |
| 391 | 403 | S | G | Y | I | P | A | N | T | T | I | P | F | G | 2300 |
| 452 | 464 | Y | Y | M | L | R | K | R | R | K | R | L | P | Y | 2500 |
| 339 | 351 | I | E | L | Q | T | I | T | P | S | T | Y | T | T | 2620 |
| 385 | 397 | V | P | S | T | S | L | S | G | Y | I | P | A | N | 2800 |
| 67 | 79 | G | G | R | T | G | Y | I | P | L | G | T | R | P | 3100 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|-------|
| 91 | 103 | P | P | L | T | V | D | P | V | G | P | S | D | P | 3300 |
| 220 | 232 | R | P | V | A | R | L | G | L | Y | S | R | T | T | 3600 |
| 34 | 46 | P | K | V | E | G | K | T | I | A | E | Q | I | L | 3650 |
| 53 | 65 | V | F | F | G | G | L | G | I | G | T | G | S | G | 3720 |
| 248 | 260 | K | L | I | T | Y | D | N | P | A | Y | E | G | I | 4050 |
| 225 | 237 | L | G | L | Y | S | R | T | T | Q | Q | V | K | V | 4100 |
| 264 | 276 | N | T | L | Y | F | S | S | N | D | N | S | I | N | 4200 |
| 50 | 62 | S | M | G | V | F | F | G | G | L | G | I | G | T | 4550 |
| 31 | 43 | D | I | I | P | K | V | E | G | K | T | I | A | E | 4950 |
| 416 | 428 | I | P | I | N | I | T | D | Q | A | P | S | L | I | 5000 |
| 451 | 463 | S | Y | Y | M | L | R | K | R | R | K | R | L | P | 5600 |
| 326 | 338 | Y | Y | Y | D | L | S | T | I | D | P | A | E | E | 6300 |
| 39 | 51 | K | T | I | A | E | Q | I | L | Q | Y | G | S | M | 6700 |
| 87 | 99 | A | P | V | R | P | P | L | T | V | D | P | V | G | 6850 |
| 364 | 376 | G | L | Y | D | I | Y | A | D | D | F | I | T | D | 7300 |
| 127 | 139 | P | P | D | V | S | G | F | S | I | T | T | S | T | 7400 |
| 258 | 270 | E | G | I | D | V | D | N | T | L | Y | F | S | S | 7500 |
| 299 | 311 | T | G | I | R | Y | S | R | I | G | N | K | Q | T | 7650 |
| 435 | 447 | P | Q | Y | T | I | I | A | D | A | G | D | F | Y | 7750 |
| 322 | 334 | A | K | V | H | Y | Y | Y | D | L | S | T | I | D | 7800 |
| 30 | 42 | P | D | I | I | P | K | V | E | G | K | T | I | A | 8000 |
| 426 | 438 | S | L | I | P | I | V | P | G | S | P | Q | Y | T | 8900 |
| 63 | 75 | G | S | G | T | G | G | R | T | G | Y | I | P | L | 9450 |
| 315 | 327 | R | S | G | K | S | I | G | A | K | V | H | Y | Y | 10200 |
| 346 | 358 | P | S | T | Y | T | T | T | S | H | A | A | S | P | 10200 |
| 93 | 105 | L | T | V | D | P | V | G | P | S | D | P | S | I | 11000 |
| 250 | 262 | I | T | Y | D | N | P | A | Y | E | G | I | D | V | 11000 |
| 304 | 316 | S | R | I | G | N | K | Q | T | L | R | T | R | S | 11000 |
| 331 | 343 | S | T | I | D | P | A | E | E | I | E | L | Q | T | 13500 |
| 186 | 198 | S | T | I | S | T | H | N | Y | E | E | I | P | M | 14000 |
| 179 | 191 | G | H | F | T | L | S | S | S | T | I | S | T | H | 14200 |
| 222 | 234 | V | A | R | L | G | L | Y | S | R | T | T | Q | Q | 15500 |
| 438 | 450 | T | I | I | A | D | A | G | D | F | Y | L | H | P | 15500 |
| 349 | 361 | Y | T | T | T | S | H | A | A | S | P | T | S | I | 16000 |
| 44 | 56 | Q | I | L | Q | Y | G | S | M | G | V | F | F | G | 18000 |
| 147 | 159 | D | I | N | N | T | V | T | T | V | T | T | H | N | 18000 |
| 247 | 259 | T | K | L | I | T | Y | D | N | P | A | Y | E | G | 18200 |
| 284 | 296 | L | D | I | V | A | L | H | R | P | A | L | T | S | 19000 |
| 180 | 192 | H | F | T | L | S | S | S | T | I | S | T | H | N | 21000 |
| 374 | 386 | I | T | D | T | S | T | T | P | V | P | S | V | P | 21000 |
| 437 | 449 | Y | T | I | I | A | D | A | G | D | F | Y | L | H | 21200 |
| 153 | 165 | T | T | V | T | T | H | N | N | P | T | F | T | D | 22200 |
| 46 | 58 | L | Q | Y | G | S | M | G | V | F | F | G | G | L | 23000 |
| 372 | 384 | D | F | I | T | D | T | S | T | T | P | V | P | S | 24000 |
| 397 | 409 | N | T | T | I | P | F | G | G | A | Y | N | I | P | 25000 |
| 317 | 329 | G | K | S | I | G | A | K | V | H | Y | Y | Y | D | 25200 |
| 429 | 441 | P | I | V | P | G | S | P | Q | Y | T | I | I | A | 25500 |
| 61 | 73 | G | T | G | S | G | T | G | G | R | T | G | Y | I | 26200 |
| 56 | 68 | G | G | L | G | I | G | T | G | S | G | T | G | G | 28000 |
| 211 | 223 | T | S | S | T | P | I | P | G | S | R | P | V | A | 29500 |
| 228 | 240 | Y | S | R | T | T | Q | Q | V | K | V | V | D | P | 32000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 453 | 465 | Y | M | L | R | K | R | R | K | R | L | P | Y | F | 33000 |
| 352 | 364 | T | S | H | A | A | S | P | T | S | I | N | N | G | 37200 |
| 283 | 295 | F | L | D | I | V | A | L | H | R | P | A | L | T | 38500 |
| 152 | 164 | V | T | T | V | T | T | H | N | N | P | T | F | T | 39000 |
| 178 | 190 | G | G | H | F | T | L | S | S | S | T | I | S | T | 39000 |
| 195 | 207 | E | I | P | M | D | T | F | I | V | S | T | N | P | 40000 |
| 143 | 155 | P | A | I | L | D | I | N | N | T | V | T | T | V | 42000 |
| 409 | 421 | P | L | V | S | G | P | D | I | P | I | N | I | T | 43000 |
| 57 | 69 | G | L | G | I | G | T | G | S | G | T | G | G | R | 44500 |
| 384 | 396 | S | V | P | S | T | S | L | S | G | Y | I | P | A | 45000 |
| 82 | 94 | A | T | D | T | L | A | P | V | R | P | P | L | T | 46000 |
| 3 | 15 | H | K | R | S | A | K | R | T | K | R | A | S | A | 46500 |
| 359 | 371 | T | S | I | N | N | G | L | Y | D | I | Y | A | D | 47200 |
| 345 | 357 | T | P | S | T | Y | T | T | T | S | H | A | A | S | 50500 |
| 214 | 226 | T | P | I | P | G | S | R | P | V | A | R | L | G | 51500 |
| 166 | 178 | P | S | V | L | Q | P | P | T | P | A | E | T | G | 53200 |
| 328 | 340 | Y | D | L | S | T | I | D | P | A | E | E | I | E | 58000 |
| 118 | 130 | G | A | P | T | S | V | P | S | I | P | P | D | V | 65200 |
| 201 | 213 | F | I | V | S | T | N | P | N | T | V | T | S | S | 65500 |
| 402 | 414 | F | G | G | A | Y | N | I | P | L | V | S | G | P | 67000 |
| 159 | 171 | N | N | P | T | F | T | D | P | S | V | L | Q | P | 69500 |
| 172 | 184 | P | T | P | A | E | T | G | G | H | F | T | L | S | 71000 |
| 37 | 49 | E | G | K | T | I | A | E | Q | I | L | Q | Y | G | 73200 |
| 136 | 148 | T | T | S | T | D | T | T | P | A | I | L | D | I | 74000 |
| 114 | 126 | F | I | D | A | G | A | P | T | S | V | P | S | I | 75000 |
| 113 | 125 | S | F | I | D | A | G | A | P | T | S | V | P | S | 76000 |
| 377 | 389 | T | S | T | T | P | V | P | S | V | P | S | T | S | 76200 |
| 286 | 298 | I | V | A | L | H | R | P | A | L | T | S | R | R | 78500 |
| 81 | 93 | T | A | T | D | T | L | A | P | V | R | P | P | L | 80000 |
| 78 | 90 | R | P | P | T | A | T | D | T | L | A | P | V | R | 86000 |
| 216 | 228 | I | P | G | S | R | P | V | A | R | L | G | L | Y | 88500 |
| 96 | 108 | D | P | V | G | P | S | D | P | S | I | V | S | L | 95000 |
| 300 | 312 | G | I | R | Y | S | R | I | G | N | K | Q | T | L | 96000 |
| 249 | 261 | L | I | T | Y | D | N | P | A | Y | E | G | I | D | 96200 |
| 107 | 119 | S | L | V | E | E | T | S | F | I | D | A | G | A | 102000 |
| 356 | 368 | A | S | P | T | S | I | N | N | G | L | Y | D | I | 110000 |
| 7 | 19 | A | K | R | T | K | R | A | S | A | T | Q | L | Y | 112000 |
| 294 | 306 | L | T | S | R | R | T | G | I | R | Y | S | R | I | 112000 |
| 106 | 118 | V | S | L | V | E | E | T | S | F | I | D | A | G | 115000 |
| 268 | 280 | F | S | S | N | D | N | S | I | N | I | A | P | D | 122000 |
| 382 | 394 | V | P | S | V | P | S | T | S | L | S | G | Y | I | 122000 |
| 419 | 431 | N | I | T | D | Q | A | P | S | L | I | P | I | V | 122000 |
| 16 | 28 | T | Q | L | Y | K | T | C | K | Q | A | G | T | C | 125000 |
| 244 | 256 | T | T | P | T | K | L | I | T | Y | D | N | P | A | 125000 |
| 18 | 30 | L | Y | K | T | C | K | Q | A | G | T | C | P | P | 130000 |
| 353 | 365 | S | H | A | A | S | P | T | S | I | N | N | G | L | 130000 |
| 109 | 121 | V | E | E | T | S | F | I | D | A | G | A | P | T | 140000 |
| 395 | 407 | P | A | N | T | T | I | P | F | G | G | A | Y | N | 140000 |
| 341 | 353 | L | Q | T | I | T | P | S | T | Y | T | T | T | S | 142000 |
| 42 | 54 | A | E | Q | I | L | Q | Y | G | S | M | G | V | F | 145000 |
| 202 | 214 | I | V | S | T | N | P | N | T | V | T | S | S | T | 145000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 461 | 473 | R | L | P | Y | F | F | S | D | V | S | L | A | A | 145000 |
| 55 | 67 | F | G | G | L | G | I | G | T | G | S | G | T | G | 150000 |
| 15 | 27 | A | T | Q | L | Y | K | T | C | K | Q | A | G | T | 155000 |
| 40 | 52 | T | I | A | E | Q | I | L | Q | Y | G | S | M | G | 160000 |
| 177 | 189 | T | G | G | H | F | T | L | S | S | S | T | I | S | 162000 |
| 198 | 210 | M | D | T | F | I | V | S | T | N | P | N | T | V | 165000 |
| 336 | 348 | A | E | E | I | E | L | Q | T | I | T | P | S | T | 180000 |
| 48 | 60 | Y | G | S | M | G | V | F | F | G | G | L | G | I | 200000 |
| 371 | 383 | D | D | F | I | T | D | T | S | T | T | P | V | P | 202000 |
| 170 | 182 | Q | P | P | T | P | A | E | T | G | G | H | F | T | 205000 |
| 431 | 443 | V | P | G | S | P | Q | Y | T | I | I | A | D | A | 205000 |
| 99 | 111 | G | P | S | D | P | S | I | V | S | L | V | E | E | 210000 |
| 263 | 275 | D | N | T | L | Y | F | S | S | N | D | N | S | I | 210000 |
| 260 | 272 | I | D | V | D | N | T | L | Y | F | S | S | N | D | 215000 |
| 38 | 50 | G | K | T | I | A | E | Q | I | L | Q | Y | G | S | 220000 |
| 338 | 350 | E | I | E | L | Q | T | I | T | P | S | T | Y | T | 222000 |
| 90 | 102 | R | P | P | L | T | V | D | P | V | G | P | S | D | 235000 |
| 396 | 408 | A | N | T | T | I | P | F | G | G | A | Y | N | I | 235000 |
| 146 | 158 | L | D | I | N | N | T | V | T | T | V | T | T | H | 240000 |
| 227 | 239 | L | Y | S | R | T | T | Q | Q | V | K | V | V | D | 250000 |
| 358 | 370 | P | T | S | I | N | N | G | L | Y | D | I | Y | A | 260000 |
| 191 | 203 | H | N | Y | E | E | I | P | M | D | T | F | I | V | 270000 |
| 342 | 354 | Q | T | I | T | P | S | T | Y | T | T | T | S | H | 270000 |
| 141 | 153 | T | T | P | A | I | L | D | I | N | N | T | V | T | 272000 |
| 14 | 26 | S | A | T | Q | L | Y | K | T | C | K | Q | A | G | 280000 |
| 399 | 411 | T | I | P | F | G | G | A | Y | N | I | P | L | V | 282000 |
| 423 | 435 | Q | A | P | S | L | I | P | I | V | P | G | S | P | 290000 |
| 259 | 271 | G | I | D | V | D | N | T | L | Y | F | S | S | N | 292000 |
| 105 | 117 | I | V | S | L | V | E | E | T | S | F | I | D | A | 305000 |
| 102 | 114 | D | P | S | I | V | S | L | V | E | E | T | S | F | 310000 |
| 205 | 217 | T | N | P | N | T | V | T | S | S | T | P | I | P | 315000 |
| 131 | 143 | S | G | F | S | I | T | T | S | T | D | T | T | P | 320000 |
| 379 | 391 | T | T | P | V | P | S | V | P | S | T | S | L | S | 325000 |
| 59 | 71 | G | I | G | T | G | S | G | T | G | G | R | T | G | 330000 |
| 219 | 231 | S | R | P | V | A | R | L | G | L | Y | S | R | T | 330000 |
| 296 | 308 | S | R | R | T | G | I | R | Y | S | R | I | G | N | 330000 |
| 279 | 291 | P | D | P | D | F | L | D | I | V | A | L | H | R | 345000 |
| 420 | 432 | I | T | D | Q | A | P | S | L | I | P | I | V | P | 345000 |
| 348 | 360 | T | Y | T | T | T | S | H | A | A | S | P | T | S | 355000 |
| 110 | 122 | E | E | T | S | F | I | D | A | G | A | P | T | S | 370000 |
| 41 | 53 | I | A | E | Q | I | L | Q | Y | G | S | M | G | V | 375000 |
| 207 | 219 | P | N | T | V | T | S | S | T | P | I | P | G | S | 375000 |
| 72 | 84 | Y | I | P | L | G | T | R | P | P | T | A | T | D | 390000 |
| 414 | 426 | P | D | I | P | I | N | I | T | D | Q | A | P | S | 390000 |
| 376 | 388 | D | T | S | T | T | P | V | P | S | V | P | S | T | 392000 |
| 446 | 458 | F | Y | L | H | P | S | Y | Y | M | L | R | K | R | 392000 |
| 280 | 292 | D | P | D | F | L | D | I | V | A | L | H | R | P | 395000 |
| 160 | 172 | N | P | T | F | T | D | P | S | V | L | Q | P | P | 410000 |
| 185 | 197 | S | S | T | I | S | T | H | N | Y | E | E | I | P | 410000 |
| 324 | 336 | V | H | Y | Y | Y | D | L | S | T | I | D | P | A | 412000 |
| 363 | 375 | N | G | L | Y | D | I | Y | A | D | D | F | I | T | 432000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 6 | 18 | S | A | K | R | T | K | R | A | S | A | T | Q | L | 455000 |
| 269 | 281 | S | S | N | D | N | S | I | N | I | A | P | D | P | 465000 |
| 132 | 144 | G | F | S | I | T | T | S | T | D | T | T | P | A | 475000 |
| 142 | 154 | T | P | A | I | L | D | I | N | N | T | V | T | T | 475000 |
| 236 | 248 | K | V | V | D | P | A | F | V | T | T | P | T | K | 480000 |
| 403 | 415 | G | G | A | Y | N | I | P | L | V | S | G | P | D | 480000 |
| 183 | 195 | L | S | S | S | T | I | S | T | H | N | Y | E | E | 485000 |
| 381 | 393 | P | V | P | S | V | P | S | T | S | L | S | G | Y | 492000 |
| 111 | 123 | E | T | S | F | I | D | A | G | A | P | T | S | V | 510000 |
| 45 | 57 | I | L | Q | Y | G | S | M | G | V | F | F | G | G | 542000 |
| 433 | 445 | G | S | P | Q | Y | T | I | I | A | D | A | G | D | 545000 |
| 246 | 258 | P | T | K | L | I | T | Y | D | N | P | A | Y | E | 562000 |
| 11 | 23 | K | R | A | S | A | T | Q | L | Y | K | T | C | K | 570000 |
| 128 | 140 | P | D | V | S | G | F | S | I | T | T | S | T | D | 585000 |
| 217 | 229 | P | G | S | R | P | V | A | R | L | G | L | Y | S | 635000 |
| 267 | 279 | Y | F | S | S | N | D | N | S | I | N | I | A | P | 642000 |
| 121 | 133 | T | S | V | P | S | I | P | P | D | V | S | G | F | 655000 |
| 98 | 110 | V | G | P | S | D | P | S | I | V | S | L | V | E | 670000 |
| 193 | 205 | Y | E | E | I | P | M | D | T | F | I | V | S | T | 710000 |
| 230 | 242 | R | T | T | Q | Q | V | K | V | V | D | P | A | F | 710000 |
| 354 | 366 | H | A | A | S | P | T | S | I | N | N | G | L | Y | 820000 |
| 370 | 382 | A | D | D | F | I | T | D | T | S | T | T | P | V | 842000 |
| 406 | 418 | Y | N | I | P | L | V | S | G | P | D | I | P | I | 855000 |
| 392 | 404 | G | Y | I | P | A | N | T | T | I | P | F | G | G | 870000 |
| 367 | 379 | D | I | Y | A | D | D | F | I | T | D | T | S | T | 910000 |
| 138 | 150 | S | T | D | T | T | P | A | I | L | D | I | N | N | 930000 |
| 307 | 319 | G | N | K | Q | T | L | R | T | R | S | G | K | S | 965000 |
| 190 | 202 | T | H | N | Y | E | E | I | P | M | D | T | F | I | 985000 |
| 1 | 13 | M | R | H | K | R | S | A | K | R | T | K | R | A | 1000000 |
| 2 | 14 | R | H | K | R | S | A | K | R | T | K | R | A | S | 1000000 |
| 4 | 16 | K | R | S | A | K | R | T | K | R | A | S | A | T | 1000000 |
| 5 | 17 | R | S | A | K | R | T | K | R | A | S | A | T | Q | 1000000 |
| 8 | 20 | K | R | T | K | R | A | S | A | T | Q | L | Y | K | 1000000 |
| 9 | 21 | R | T | K | R | A | S | A | T | Q | L | Y | K | T | 1000000 |
| 10 | 22 | T | K | R | A | S | A | T | Q | L | Y | K | T | C | 1000000 |
| 12 | 24 | R | A | S | A | T | Q | L | Y | K | T | C | K | Q | 1000000 |
| 13 | 25 | A | S | A | T | Q | L | Y | K | T | C | K | Q | A | 1000000 |
| 19 | 31 | Y | K | T | C | K | Q | A | G | T | C | P | P | D | 1000000 |
| 20 | 32 | K | T | C | K | Q | A | G | T | C | P | P | D | I | 1000000 |
| 21 | 33 | T | C | K | Q | A | G | T | C | P | P | D | I | I | 1000000 |
| 22 | 34 | C | K | Q | A | G | T | C | P | P | D | I | I | P | 1000000 |
| 23 | 35 | K | Q | A | G | T | C | P | P | D | I | I | P | K | 1000000 |
| 24 | 36 | Q | A | G | T | C | P | P | D | I | I | P | K | V | 1000000 |
| 25 | 37 | A | G | T | C | P | P | D | I | I | P | K | V | E | 1000000 |
| 26 | 38 | G | T | C | P | P | D | I | I | P | K | V | E | G | 1000000 |
| 27 | 39 | T | C | P | P | D | I | I | P | K | V | E | G | K | 1000000 |
| 28 | 40 | C | P | P | D | I | I | P | K | V | E | G | K | T | 1000000 |
| 29 | 41 | P | P | D | I | I | P | K | V | E | G | K | T | I | 1000000 |
| 32 | 44 | I | I | P | K | V | E | G | K | T | I | A | E | Q | 1000000 |
| 33 | 45 | I | P | K | V | E | G | K | T | I | A | E | Q | I | 1000000 |
| 35 | 47 | K | V | E | G | K | T | I | A | E | Q | I | L | Q | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 36 | 48 | V | E | G | K | T | I | A | E | Q | I | L | Q | Y | 1000000 |
| 47 | 59 | Q | Y | G | S | M | G | V | F | F | G | G | L | G | 1000000 |
| 54 | 66 | F | F | G | G | L | G | I | G | T | G | S | G | T | 1000000 |
| 58 | 70 | L | G | I | G | T | G | S | G | T | G | G | R | T | 1000000 |
| 60 | 72 | I | G | T | G | S | G | T | G | G | R | T | G | Y | 1000000 |
| 62 | 74 | T | G | S | G | T | G | G | R | T | G | Y | I | P | 1000000 |
| 64 | 76 | S | G | T | G | G | R | T | G | Y | I | P | L | G | 1000000 |
| 65 | 77 | G | T | G | G | R | T | G | Y | I | P | L | G | T | 1000000 |
| 66 | 78 | T | G | G | R | T | G | Y | I | P | L | G | T | R | 1000000 |
| 68 | 80 | G | R | T | G | Y | I | P | L | G | T | R | P | P | 1000000 |
| 69 | 81 | R | T | G | Y | I | P | L | G | T | R | P | P | T | 1000000 |
| 71 | 83 | G | Y | I | P | L | G | T | R | P | P | T | A | T | 1000000 |
| 73 | 85 | I | P | L | G | T | R | P | P | T | A | T | D | T | 1000000 |
| 74 | 86 | P | L | G | T | R | P | P | T | A | T | D | T | L | 1000000 |
| 75 | 87 | L | G | T | R | P | P | T | A | T | D | T | L | A | 1000000 |
| 76 | 88 | G | T | R | P | P | T | A | T | D | T | L | A | P | 1000000 |
| 77 | 89 | T | R | P | P | T | A | T | D | T | L | A | P | V | 1000000 |
| 79 | 91 | P | P | T | A | T | D | T | L | A | P | V | R | P | 1000000 |
| 80 | 92 | P | T | A | T | D | T | L | A | P | V | R | P | P | 1000000 |
| 83 | 95 | T | D | T | L | A | P | V | R | P | P | L | T | V | 1000000 |
| 85 | 97 | T | L | A | P | V | R | P | P | L | T | V | D | P | 1000000 |
| 86 | 98 | L | A | P | V | R | P | P | L | T | V | D | P | V | 1000000 |
| 88 | 100 | P | V | R | P | P | L | T | V | D | P | V | G | P | 1000000 |
| 89 | 101 | V | R | P | P | L | T | V | D | P | V | G | P | S | 1000000 |
| 92 | 104 | P | L | T | V | D | P | V | G | P | S | D | P | S | 1000000 |
| 94 | 106 | T | V | D | P | V | G | P | S | D | P | S | I | V | 1000000 |
| 95 | 107 | V | D | P | V | G | P | S | D | P | S | I | V | S | 1000000 |
| 97 | 109 | P | V | G | P | S | D | P | S | I | V | S | L | V | 1000000 |
| 100 | 112 | P | S | D | P | S | I | V | S | L | V | E | E | T | 1000000 |
| 101 | 113 | S | D | P | S | I | V | S | L | V | E | E | T | S | 1000000 |
| 104 | 116 | S | I | V | S | L | V | E | E | T | S | F | I | D | 1000000 |
| 108 | 120 | L | V | E | E | T | S | F | I | D | A | G | A | P | 1000000 |
| 115 | 127 | I | D | A | G | A | P | T | S | V | P | S | I | P | 1000000 |
| 116 | 128 | D | A | G | A | P | T | S | V | P | S | I | P | P | 1000000 |
| 117 | 129 | A | G | A | P | T | S | V | P | S | I | P | P | D | 1000000 |
| 119 | 131 | A | P | T | S | V | P | S | I | P | P | D | V | S | 1000000 |
| 120 | 132 | P | T | S | V | P | S | I | P | P | D | V | S | G | 1000000 |
| 122 | 134 | S | V | P | S | I | P | P | D | V | S | G | F | S | 1000000 |
| 123 | 135 | V | P | S | I | P | P | D | V | S | G | F | S | I | 1000000 |
| 124 | 136 | P | S | I | P | P | D | V | S | G | F | S | I | T | 1000000 |
| 125 | 137 | S | I | P | P | D | V | S | G | F | S | I | T | T | 1000000 |
| 126 | 138 | I | P | P | D | V | S | G | F | S | I | T | T | S | 1000000 |
| 129 | 141 | D | V | S | G | F | S | I | T | T | S | T | D | T | 1000000 |
| 130 | 142 | V | S | G | F | S | I | T | T | S | T | D | T | T | 1000000 |
| 133 | 145 | F | S | I | T | T | S | T | D | T | T | P | A | I | 1000000 |
| 134 | 146 | S | I | T | T | S | T | D | T | T | P | A | I | L | 1000000 |
| 135 | 147 | I | T | T | S | T | D | T | T | P | A | I | L | D | 1000000 |
| 137 | 149 | T | S | T | D | T | T | P | A | I | L | D | I | N | 1000000 |
| 139 | 151 | T | D | T | T | P | A | I | L | D | I | N | N | T | 1000000 |
| 140 | 152 | D | T | T | P | A | I | L | D | I | N | N | T | V | 1000000 |
| 145 | 157 | I | L | D | I | N | N | T | V | T | T | V | T | T | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 148 | 160 | I | N | N | T | V | T | T | V | T | T | H | N | N | 1000000 |
| 149 | 161 | N | N | T | V | T | T | V | T | T | H | N | N | P | 1000000 |
| 151 | 163 | T | V | T | T | V | T | T | H | N | N | P | T | F | 1000000 |
| 154 | 166 | T | V | T | T | H | N | N | P | T | F | T | D | P | 1000000 |
| 155 | 167 | V | T | T | H | N | N | P | T | F | T | D | P | S | 1000000 |
| 156 | 168 | T | T | H | N | N | P | T | F | T | D | P | S | V | 1000000 |
| 157 | 169 | T | H | N | N | P | T | F | T | D | P | S | V | L | 1000000 |
| 158 | 170 | H | N | N | P | T | F | T | D | P | S | V | L | Q | 1000000 |
| 162 | 174 | T | F | T | D | P | S | V | L | Q | P | P | T | P | 1000000 |
| 163 | 175 | F | T | D | P | S | V | L | Q | P | P | T | P | A | 1000000 |
| 164 | 176 | T | D | P | S | V | L | Q | P | P | T | P | A | E | 1000000 |
| 165 | 177 | D | P | S | V | L | Q | P | P | T | P | A | E | T | 1000000 |
| 168 | 180 | V | L | Q | P | P | T | P | A | E | T | G | G | H | 1000000 |
| 169 | 181 | L | Q | P | P | T | P | A | E | T | G | G | H | F | 1000000 |
| 171 | 183 | P | P | T | P | A | E | T | G | G | H | F | T | L | 1000000 |
| 173 | 185 | T | P | A | E | T | G | G | H | F | T | L | S | S | 1000000 |
| 174 | 186 | P | A | E | T | G | G | H | F | T | L | S | S | S | 1000000 |
| 175 | 187 | A | E | T | G | G | H | F | T | L | S | S | S | T | 1000000 |
| 176 | 188 | E | T | G | G | H | F | T | L | S | S | S | T | I | 1000000 |
| 182 | 194 | T | L | S | S | S | T | I | S | T | H | N | Y | E | 1000000 |
| 184 | 196 | S | S | S | T | I | S | T | H | N | Y | E | E | I | 1000000 |
| 187 | 199 | T | I | S | T | H | N | Y | E | E | I | P | M | D | 1000000 |
| 188 | 200 | I | S | T | H | N | Y | E | E | I | P | M | D | T | 1000000 |
| 189 | 201 | S | T | H | N | Y | E | E | I | P | M | D | T | F | 1000000 |
| 192 | 204 | N | Y | E | E | I | P | M | D | T | F | I | V | S | 1000000 |
| 194 | 206 | E | E | I | P | M | D | T | F | I | V | S | T | N | 1000000 |
| 197 | 209 | P | M | D | T | F | I | V | S | T | N | P | N | T | 1000000 |
| 203 | 215 | V | S | T | N | P | N | T | V | T | S | S | T | P | 1000000 |
| 204 | 216 | S | T | N | P | N | T | V | T | S | S | T | P | I | 1000000 |
| 206 | 218 | N | P | N | T | V | T | S | S | T | P | I | P | G | 1000000 |
| 209 | 221 | T | V | T | S | S | T | P | I | P | G | S | R | P | 1000000 |
| 210 | 222 | V | T | S | S | T | P | I | P | G | S | R | P | V | 1000000 |
| 212 | 224 | S | S | T | P | I | P | G | S | R | P | V | A | R | 1000000 |
| 213 | 225 | S | T | P | I | P | G | S | R | P | V | A | R | L | 1000000 |
| 215 | 227 | P | I | P | G | S | R | P | V | A | R | L | G | L | 1000000 |
| 218 | 230 | G | S | R | P | V | A | R | L | G | L | Y | S | R | 1000000 |
| 221 | 233 | P | V | A | R | L | G | L | Y | S | R | T | T | Q | 1000000 |
| 223 | 235 | A | R | L | G | L | Y | S | R | T | T | Q | Q | V | 1000000 |
| 224 | 236 | R | L | G | L | Y | S | R | T | T | Q | Q | V | K | 1000000 |
| 229 | 241 | S | R | T | T | Q | Q | V | K | V | V | D | P | A | 1000000 |
| 231 | 243 | T | T | Q | Q | V | K | V | V | D | P | A | F | V | 1000000 |
| 232 | 244 | T | Q | Q | V | K | V | V | D | P | A | F | V | T | 1000000 |
| 234 | 246 | Q | V | K | V | V | D | P | A | F | V | T | T | P | 1000000 |
| 237 | 249 | V | V | D | P | A | F | V | T | T | P | T | K | L | 1000000 |
| 238 | 250 | V | D | P | A | F | V | T | T | P | T | K | L | I | 1000000 |
| 239 | 251 | D | P | A | F | V | T | T | P | T | K | L | I | T | 1000000 |
| 242 | 254 | F | V | T | T | P | T | K | L | I | T | Y | D | N | 1000000 |
| 243 | 255 | V | T | T | P | T | K | L | I | T | Y | D | N | P | 1000000 |
| 245 | 257 | T | P | T | K | L | I | T | Y | D | N | P | A | Y | 1000000 |
| 251 | 263 | T | Y | D | N | P | A | Y | E | G | I | D | V | D | 1000000 |
| 252 | 264 | Y | D | N | P | A | Y | E | G | I | D | V | D | N | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 253 | 265 | D | N | P | A | Y | E | G | I | D | V | D | N | T | 1000000 |
| 254 | 266 | N | P | A | Y | E | G | I | D | V | D | N | T | L | 1000000 |
| 256 | 268 | A | Y | E | G | I | D | V | D | N | T | L | Y | F | 1000000 |
| 257 | 269 | Y | E | G | I | D | V | D | N | T | L | Y | F | S | 1000000 |
| 261 | 273 | D | V | D | N | T | L | Y | F | S | S | N | D | N | 1000000 |
| 262 | 274 | V | D | N | T | L | Y | F | S | S | N | D | N | S | 1000000 |
| 270 | 282 | S | N | D | N | S | I | N | I | A | P | D | P | D | 1000000 |
| 271 | 283 | N | D | N | S | I | N | I | A | P | D | P | D | F | 1000000 |
| 272 | 284 | D | N | S | I | N | I | A | P | D | P | D | F | L | 1000000 |
| 273 | 285 | N | S | I | N | I | A | P | D | P | D | F | L | D | 1000000 |
| 274 | 286 | S | I | N | I | A | P | D | P | D | F | L | D | I | 1000000 |
| 275 | 287 | I | N | I | A | P | D | P | D | F | L | D | I | V | 1000000 |
| 276 | 288 | N | I | A | P | D | P | D | F | L | D | I | V | A | 1000000 |
| 277 | 289 | I | A | P | D | P | D | F | L | D | I | V | A | L | 1000000 |
| 278 | 290 | A | P | D | P | D | F | L | D | I | V | A | L | H | 1000000 |
| 287 | 299 | V | A | L | H | R | P | A | L | T | S | R | R | T | 1000000 |
| 288 | 300 | A | L | H | R | P | A | L | T | S | R | R | T | G | 1000000 |
| 289 | 301 | L | H | R | P | A | L | T | S | R | R | T | G | I | 1000000 |
| 290 | 302 | H | R | P | A | L | T | S | R | R | T | G | I | R | 1000000 |
| 291 | 303 | R | P | A | L | T | S | R | R | T | G | I | R | Y | 1000000 |
| 293 | 305 | A | L | T | S | R | R | T | G | I | R | Y | S | R | 1000000 |
| 295 | 307 | T | S | R | R | T | G | I | R | Y | S | R | I | G | 1000000 |
| 297 | 309 | R | R | T | G | I | R | Y | S | R | I | G | N | K | 1000000 |
| 298 | 310 | R | T | G | I | R | Y | S | R | I | G | N | K | Q | 1000000 |
| 302 | 314 | R | Y | S | R | I | G | N | K | Q | T | L | R | T | 1000000 |
| 303 | 315 | Y | S | R | I | G | N | K | Q | T | L | R | T | R | 1000000 |
| 305 | 317 | R | I | G | N | K | Q | T | L | R | T | R | S | G | 1000000 |
| 306 | 318 | I | G | N | K | Q | T | L | R | T | R | S | G | K | 1000000 |
| 308 | 320 | N | K | Q | T | L | R | T | R | S | G | K | S | I | 1000000 |
| 309 | 321 | K | Q | T | L | R | T | R | S | G | K | S | I | G | 1000000 |
| 311 | 323 | T | L | R | T | R | S | G | K | S | I | G | A | K | 1000000 |
| 312 | 324 | L | R | T | R | S | G | K | S | I | G | A | K | V | 1000000 |
| 313 | 325 | R | T | R | S | G | K | S | I | G | A | K | V | H | 1000000 |
| 314 | 326 | T | R | S | G | K | S | I | G | A | K | V | H | Y | 1000000 |
| 316 | 328 | S | G | K | S | I | G | A | K | V | H | Y | Y | Y | 1000000 |
| 318 | 330 | K | S | I | G | A | K | V | H | Y | Y | Y | Y | D | 1000000 |
| 319 | 331 | S | I | G | A | K | V | H | Y | Y | Y | Y | D | L | 1000000 |
| 320 | 332 | I | G | A | K | V | H | Y | Y | Y | Y | D | L | S | 1000000 |
| 321 | 333 | G | A | K | V | H | Y | Y | Y | Y | D | L | S | T | 1000000 |
| 323 | 335 | K | V | H | Y | Y | Y | D | L | S | T | I | D | P | 1000000 |
| 327 | 339 | Y | Y | D | L | S | T | I | D | P | A | E | E | I | 1000000 |
| 329 | 341 | D | L | S | T | I | D | P | A | E | E | I | E | L | 1000000 |
| 330 | 342 | L | S | T | I | D | P | A | E | E | I | E | L | Q | 1000000 |
| 332 | 344 | T | I | D | P | A | E | E | I | E | L | Q | T | I | 1000000 |
| 333 | 345 | I | D | P | A | E | E | I | E | L | Q | T | I | T | 1000000 |
| 334 | 346 | D | P | A | E | E | I | E | L | Q | T | I | T | P | 1000000 |
| 335 | 347 | P | A | E | E | I | E | L | Q | T | I | T | P | S | 1000000 |
| 337 | 349 | E | E | I | E | L | Q | T | I | T | P | S | T | Y | 1000000 |
| 340 | 352 | E | L | Q | T | I | T | P | S | T | Y | T | T | T | 1000000 |
| 343 | 355 | T | I | T | P | S | T | Y | T | T | T | S | H | A | 1000000 |
| 344 | 356 | I | T | P | S | T | Y | T | T | T | S | H | A | A | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 350 | 362 | T | T | T | S | H | A | A | S | P | T | S | I | N | 1000000 |
| 351 | 363 | T | T | S | H | A | A | S | P | T | S | I | N | N | 1000000 |
| 355 | 367 | A | A | S | P | T | S | I | N | N | G | L | Y | D | 1000000 |
| 357 | 369 | S | P | T | S | I | N | N | G | L | Y | D | I | Y | 1000000 |
| 360 | 372 | S | I | N | N | G | L | Y | D | I | Y | A | D | D | 1000000 |
| 361 | 373 | I | N | N | G | L | Y | D | I | Y | A | D | D | F | 1000000 |
| 362 | 374 | N | N | G | L | Y | D | I | Y | A | D | D | F | I | 1000000 |
| 365 | 377 | L | Y | D | I | Y | A | D | D | F | I | T | D | T | 1000000 |
| 366 | 378 | Y | D | I | Y | A | D | D | F | I | T | D | T | S | 1000000 |
| 368 | 380 | I | Y | A | D | D | F | I | T | D | T | S | T | T | 1000000 |
| 369 | 381 | Y | A | D | D | F | I | T | D | T | S | T | T | P | 1000000 |
| 373 | 385 | F | I | T | D | T | S | T | T | P | V | P | S | V | 1000000 |
| 375 | 387 | T | D | T | S | T | T | P | V | P | S | V | P | S | 1000000 |
| 378 | 390 | S | T | T | P | V | P | S | V | P | S | T | S | L | 1000000 |
| 380 | 392 | T | P | V | P | S | V | P | S | T | S | L | S | G | 1000000 |
| 383 | 395 | P | S | V | P | S | T | S | L | S | G | Y | I | P | 1000000 |
| 386 | 398 | P | S | T | S | L | S | G | Y | I | P | A | N | T | 1000000 |
| 387 | 399 | S | T | S | L | S | G | Y | I | P | A | N | T | T | 1000000 |
| 389 | 401 | S | L | S | G | Y | I | P | A | N | T | T | I | P | 1000000 |
| 390 | 402 | L | S | G | Y | I | P | A | N | T | T | I | P | F | 1000000 |
| 393 | 405 | Y | I | P | A | N | T | T | I | P | F | G | G | A | 1000000 |
| 394 | 406 | I | P | A | N | T | T | I | P | F | G | G | A | Y | 1000000 |
| 401 | 413 | P | F | G | G | A | Y | N | I | P | L | V | S | G | 1000000 |
| 405 | 417 | A | Y | N | I | P | L | V | S | G | P | D | I | P | 1000000 |
| 407 | 419 | N | I | P | L | V | S | G | P | D | I | P | I | N | 1000000 |
| 410 | 422 | L | V | S | G | P | D | I | P | I | N | I | T | D | 1000000 |
| 411 | 423 | V | S | G | P | D | I | P | I | N | I | T | D | Q | 1000000 |
| 412 | 424 | S | G | P | D | I | P | I | N | I | T | D | Q | A | 1000000 |
| 413 | 425 | G | P | D | I | P | I | N | I | T | D | Q | A | P | 1000000 |
| 415 | 427 | D | I | P | I | N | I | T | D | Q | A | P | S | L | 1000000 |
| 417 | 429 | P | I | N | I | T | D | Q | A | P | S | L | I | P | 1000000 |
| 421 | 433 | T | D | Q | A | P | S | L | I | P | I | V | P | G | 1000000 |
| 422 | 434 | D | Q | A | P | S | L | I | P | I | V | P | G | S | 1000000 |
| 424 | 436 | A | P | S | L | I | P | I | V | P | G | S | P | Q | 1000000 |
| 427 | 439 | L | I | P | I | V | P | G | S | P | Q | Y | T | I | 1000000 |
| 430 | 442 | I | V | P | G | S | P | Q | Y | T | I | I | A | D | 1000000 |
| 432 | 444 | P | G | S | P | Q | Y | T | I | I | A | D | A | G | 1000000 |
| 434 | 446 | S | P | Q | Y | T | I | I | A | D | A | G | D | F | 1000000 |
| 436 | 448 | Q | Y | T | I | I | A | D | A | G | D | F | Y | L | 1000000 |
| 439 | 451 | I | I | A | D | A | G | D | F | Y | L | H | P | S | 1000000 |
| 440 | 452 | I | A | D | A | G | D | F | Y | L | H | P | S | Y | 1000000 |
| 441 | 453 | A | D | A | G | D | F | Y | L | H | P | S | Y | Y | 1000000 |
| 442 | 454 | D | A | G | D | F | Y | L | H | P | S | Y | Y | M | 1000000 |
| 443 | 455 | A | G | D | F | Y | L | H | P | S | Y | Y | M | L | 1000000 |
| 447 | 459 | Y | L | H | P | S | Y | Y | M | L | R | K | R | R | 1000000 |
| 448 | 460 | L | H | P | S | Y | Y | M | L | R | K | R | R | K | 1000000 |
| 449 | 461 | H | P | S | Y | Y | M | L | R | K | R | R | K | R | 1000000 |
| 454 | 466 | M | L | R | K | R | R | K | R | L | P | Y | F | F | 1000000 |
| 455 | 467 | L | R | K | R | R | K | R | L | P | Y | F | F | S | 1000000 |
| 456 | 468 | R | K | R | R | K | R | L | P | Y | F | F | S | D | 1000000 |
| 457 | 469 | K | R | R | K | R | L | P | Y | F | F | S | D | V | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 458 | 470 | R | R | K | R | L | P | Y | F | F | S | D | V | S | 1000000 |
| 459 | 471 | R | K | R | L | P | Y | F | F | S | D | V | S | L | 1000000 |
| 460 | 472 | K | R | L | P | Y | F | F | S | D | V | S | L | A | 1000000 |

| | | | | | | | | | | | | | | | IC50 DRB1*0401 nM) |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------------------|
| 46 | 58 | L | Q | Y | G | S | M | G | V | F | F | G | G | L | 9.8 |
| 266 | 278 | L | Y | F | S | S | N | D | N | S | I | N | I | A | 11 |
| 391 | 403 | S | G | Y | I | P | A | N | T | T | I | P | F | G | 12 |
| 444 | 456 | G | D | F | Y | L | H | P | S | Y | Y | M | L | R | 20.2 |
| 179 | 191 | G | H | F | T | L | S | S | S | T | I | S | T | H | 23.2 |
| 199 | 211 | D | T | F | I | V | S | T | N | P | N | T | V | T | 26 |
| 325 | 337 | H | Y | Y | Y | D | L | S | T | I | D | P | A | E | 44.5 |
| 208 | 220 | N | T | V | T | S | S | T | P | I | P | G | S | R | 62 |
| 131 | 143 | S | G | F | S | I | T | T | S | T | D | T | T | P | 66.5 |
| 347 | 359 | S | T | Y | T | T | T | S | H | A | A | S | P | T | 86 |
| 70 | 82 | T | G | Y | I | P | L | G | T | R | P | P | T | A | 91 |
| 17 | 29 | Q | L | Y | K | T | C | K | Q | A | G | T | C | P | 112 |
| 112 | 124 | T | S | F | I | D | A | G | A | P | T | S | V | P | 120 |
| 143 | 155 | P | A | I | L | D | I | N | N | T | V | T | T | V | 122 |
| 43 | 55 | E | Q | I | L | Q | Y | G | S | M | G | V | F | F | 150 |
| 326 | 338 | Y | Y | Y | D | L | S | T | I | D | P | A | E | E | 150 |
| 51 | 63 | M | G | V | F | F | G | G | L | G | I | G | T | G | 170 |
| 150 | 162 | N | T | V | T | T | V | T | T | H | N | N | P | T | 175 |
| 299 | 311 | T | G | I | R | Y | S | R | I | G | N | K | Q | T | 192 |
| 281 | 293 | P | D | F | L | D | I | V | A | L | H | R | P | A | 250 |
| 53 | 65 | V | F | F | G | G | L | G | I | G | T | G | S | G | 285 |
| 264 | 276 | N | T | L | Y | F | S | S | N | D | N | S | I | N | 305 |
| 337 | 349 | E | E | I | E | L | Q | T | I | T | P | S | T | Y | 335 |
| 52 | 64 | G | V | F | F | G | G | L | G | I | G | T | G | S | 340 |
| 200 | 212 | T | F | I | V | S | T | N | P | N | T | V | T | S | 385 |
| 372 | 384 | D | F | I | T | D | T | S | T | T | P | V | P | S | 420 |
| 388 | 400 | T | S | L | S | G | Y | I | P | A | N | T | T | I | 445 |
| 367 | 379 | D | I | Y | A | D | D | F | I | T | D | T | S | T | 450 |
| 437 | 449 | Y | T | I | I | A | D | A | G | D | F | Y | L | H | 530 |
| 181 | 193 | F | T | L | S | S | S | T | I | S | T | H | N | Y | 580 |
| 233 | 245 | Q | Q | V | K | V | V | D | P | A | F | V | T | T | 585 |
| 103 | 115 | P | S | I | V | S | L | V | E | E | T | S | F | I | 602 |
| 128 | 140 | P | D | V | S | G | F | S | I | T | T | S | T | D | 655 |
| 201 | 213 | F | I | V | S | T | N | P | N | T | V | T | S | S | 912 |
| 49 | 61 | G | S | M | G | V | F | F | G | G | L | G | I | G | 940 |
| 39 | 51 | K | T | I | A | E | Q | I | L | Q | Y | G | S | M | 1100 |
| 371 | 383 | D | D | F | I | T | D | T | S | T | T | P | V | P | 1100 |
| 301 | 313 | I | R | Y | S | R | I | G | N | K | Q | T | L | R | 1200 |
| 247 | 259 | T | K | L | I | T | Y | D | N | P | A | Y | E | G | 1300 |
| 435 | 447 | P | Q | Y | T | I | I | A | D | A | G | D | F | Y | 1400 |
| 324 | 336 | V | H | Y | Y | Y | D | L | S | T | I | D | P | A | 1500 |
| 346 | 358 | P | S | T | Y | T | T | T | S | H | A | A | S | P | 1650 |
| 400 | 412 | I | P | F | G | G | A | Y | N | I | P | L | V | S | 1700 |
| 285 | 297 | D | I | V | A | L | H | R | P | A | L | T | S | R | 1800 |
| 265 | 277 | T | L | Y | F | S | S | N | D | N | S | I | N | I | 1900 |
| 416 | 428 | I | P | I | N | I | T | D | Q | A | P | S | L | I | 1900 |
| 402 | 414 | F | G | G | A | Y | N | I | P | L | V | S | G | P | 1950 |
| 248 | 260 | K | L | I | T | Y | D | N | P | A | Y | E | G | I | 2100 |
| 146 | 158 | L | D | I | N | N | T | V | T | T | V | T | T | H | 2220 |
| 445 | 457 | D | F | Y | L | H | P | S | Y | Y | M | L | R | K | 2300 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|-------|
| 255 | 267 | P | A | Y | E | G | I | D | V | D | N | T | L | Y | 2320 |
| 312 | 324 | L | R | T | R | S | G | K | S | I | G | A | K | V | 2850 |
| 425 | 437 | P | S | L | I | P | I | V | P | G | S | P | Q | Y | 3000 |
| 58 | 70 | L | G | I | G | T | G | S | G | T | G | G | R | T | 3350 |
| 284 | 296 | L | D | I | V | A | L | H | R | P | A | L | T | S | 3400 |
| 152 | 164 | V | T | T | V | T | T | H | N | N | P | T | F | T | 3620 |
| 14 | 26 | S | A | T | Q | L | Y | K | T | C | K | Q | A | G | 3700 |
| 66 | 78 | T | G | G | R | T | G | Y | I | P | L | G | T | R | 3700 |
| 397 | 409 | N | T | T | I | P | F | G | G | A | Y | N | I | P | 4000 |
| 418 | 430 | I | N | I | T | D | Q | A | P | S | L | I | P | I | 4200 |
| 203 | 215 | V | S | T | N | P | N | T | V | T | S | S | T | P | 4800 |
| 240 | 252 | P | A | F | V | T | T | P | T | K | L | I | T | Y | 4900 |
| 180 | 192 | H | F | T | L | S | S | S | T | I | S | T | H | N | 5000 |
| 161 | 173 | P | T | F | T | D | P | S | V | L | Q | P | P | T | 5220 |
| 292 | 304 | P | A | L | T | S | R | R | T | G | I | R | Y | S | 5220 |
| 106 | 118 | V | S | L | V | E | E | T | S | F | I | D | A | G | 5400 |
| 386 | 398 | P | S | T | S | L | S | G | Y | I | P | A | N | T | 5500 |
| 446 | 458 | F | Y | L | H | P | S | Y | Y | M | L | R | K | R | 5700 |
| 249 | 261 | L | I | T | Y | D | N | P | A | Y | E | G | I | D | 5850 |
| 263 | 275 | D | N | T | L | Y | F | S | S | N | D | N | S | I | 5920 |
| 153 | 165 | T | T | V | T | T | H | N | N | P | T | F | T | D | 6000 |
| 84 | 96 | D | T | L | A | P | V | R | P | P | L | T | V | D | 6200 |
| 38 | 50 | G | K | T | I | A | E | Q | I | L | Q | Y | G | S | 6400 |
| 207 | 219 | P | N | T | V | T | S | S | T | P | I | P | G | S | 6450 |
| 258 | 270 | E | G | I | D | V | D | N | T | L | Y | F | S | S | 6620 |
| 282 | 294 | D | F | L | D | I | V | A | L | H | R | P | A | L | 6800 |
| 225 | 237 | L | G | L | Y | S | R | T | T | Q | Q | V | K | V | 7000 |
| 438 | 450 | T | I | I | A | D | A | G | D | F | Y | L | H | P | 7000 |
| 259 | 271 | G | I | D | V | D | N | T | L | Y | F | S | S | N | 7150 |
| 342 | 354 | Q | T | I | T | P | S | T | Y | T | T | T | S | H | 7420 |
| 134 | 146 | S | I | T | T | S | T | D | T | T | P | A | I | L | 8000 |
| 55 | 67 | F | G | G | L | G | I | G | T | G | S | G | T | G | 8920 |
| 209 | 221 | T | V | T | S | S | T | P | I | P | G | S | R | P | 9250 |
| 135 | 147 | I | T | T | S | T | D | T | T | P | A | I | L | D | 9300 |
| 349 | 361 | Y | T | T | T | S | H | A | A | S | P | T | S | I | 9850 |
| 339 | 351 | I | E | L | Q | T | I | T | P | S | T | Y | T | T | 9950 |
| 56 | 68 | G | G | L | G | I | G | T | G | S | G | T | G | G | 11000 |
| 133 | 145 | F | S | I | T | T | S | T | D | T | T | P | A | I | 13000 |
| 268 | 280 | F | S | S | N | D | N | S | I | N | I | A | P | D | 14000 |
| 322 | 334 | A | K | V | H | Y | Y | Y | D | L | S | T | I | D | 15200 |
| 363 | 375 | N | G | L | Y | D | I | Y | A | D | D | F | I | T | 15200 |
| 8 | 20 | K | R | T | K | R | A | S | A | T | Q | L | Y | K | 17200 |
| 144 | 156 | A | I | L | D | I | N | N | T | V | T | T | V | T | 17500 |
| 154 | 166 | T | V | T | T | H | N | N | P | T | F | T | D | P | 17500 |
| 186 | 198 | S | T | I | S | T | H | N | Y | E | E | I | P | M | 17500 |
| 226 | 238 | G | L | Y | S | R | T | T | Q | Q | V | K | V | V | 17500 |
| 364 | 376 | G | L | Y | D | I | Y | A | D | D | F | I | T | D | 18000 |
| 198 | 210 | M | D | T | F | I | V | S | T | N | P | N | T | V | 18500 |
| 185 | 197 | S | S | T | I | S | T | H | N | Y | E | E | I | P | 21500 |
| 91 | 103 | P | P | L | T | V | D | P | V | G | P | S | D | P | 22000 |
| 404 | 416 | G | A | Y | N | I | P | L | V | S | G | P | D | I | 23200 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 377 | 389 | T | S | T | T | P | V | P | S | V | P | S | T | S | 23500 |
| 414 | 426 | P | D | I | P | I | N | I | T | D | Q | A | P | S | 24000 |
| 350 | 362 | T | T | T | S | H | A | A | S | P | T | S | I | N | 25500 |
| 149 | 161 | N | N | T | V | T | T | V | T | T | H | N | N | P | 26500 |
| 42 | 54 | A | E | Q | I | L | Q | Y | G | S | M | G | V | F | 27000 |
| 450 | 462 | P | S | Y | Y | M | L | R | K | R | R | K | R | L | 28200 |
| 387 | 399 | S | T | S | L | S | G | Y | I | P | A | N | T | T | 29000 |
| 331 | 343 | S | T | I | D | P | A | E | E | I | E | L | Q | T | 29500 |
| 110 | 122 | E | E | T | S | F | I | D | A | G | A | P | T | S | 30000 |
| 309 | 321 | K | Q | T | L | R | T | R | S | G | K | S | I | G | 30200 |
| 50 | 62 | S | M | G | V | F | F | G | G | L | G | I | G | T | 31000 |
| 151 | 163 | T | V | T | T | V | T | T | H | N | N | P | T | F | 39000 |
| 246 | 258 | P | T | K | L | I | T | Y | D | N | P | A | Y | E | 41000 |
| 317 | 329 | G | K | S | I | G | A | K | V | H | Y | Y | Y | D | 41000 |
| 175 | 187 | A | E | T | G | G | H | F | T | L | S | S | S | T | 41500 |
| 44 | 56 | Q | I | L | Q | Y | G | S | M | G | V | F | F | G | 43000 |
| 236 | 248 | K | V | V | D | P | A | F | V | T | T | P | T | K | 43000 |
| 224 | 236 | R | L | G | L | Y | S | R | T | T | Q | Q | V | K | 46000 |
| 220 | 232 | R | P | V | A | R | L | G | L | Y | S | R | T | T | 47000 |
| 16 | 28 | T | Q | L | Y | K | T | C | K | Q | A | G | T | C | 48200 |
| 348 | 360 | T | Y | T | T | T | S | H | A | A | S | P | T | S | 51500 |
| 273 | 285 | N | S | I | N | I | A | P | D | P | D | F | L | D | 53500 |
| 358 | 370 | P | T | S | I | N | N | G | L | Y | D | I | Y | A | 59000 |
| 113 | 125 | S | F | I | D | A | G | A | P | T | S | V | P | S | 61200 |
| 359 | 371 | T | S | I | N | N | G | L | Y | D | I | Y | A | D | 61500 |
| 431 | 443 | V | P | G | S | P | Q | Y | T | I | I | A | D | A | 65000 |
| 460 | 472 | K | R | L | P | Y | F | F | S | D | V | S | L | A | 65000 |
| 93 | 105 | L | T | V | D | P | V | G | P | S | D | P | S | I | 66000 |
| 182 | 194 | T | L | S | S | S | T | I | S | T | H | N | Y | E | 72500 |
| 423 | 435 | Q | A | P | S | L | I | P | I | V | P | G | S | P | 73000 |
| 196 | 208 | I | P | M | D | T | F | I | V | S | T | N | P | N | 74500 |
| 409 | 421 | P | L | V | S | G | P | D | I | P | I | N | I | T | 77000 |
| 433 | 445 | G | S | P | Q | Y | T | I | I | A | D | A | G | D | 78000 |
| 315 | 327 | R | S | G | K | S | I | G | A | K | V | H | Y | Y | 79000 |
| 57 | 69 | G | L | G | I | G | T | G | S | G | T | G | G | R | 87000 |
| 374 | 386 | I | T | D | T | S | T | T | P | V | P | S | V | P | 87200 |
| 428 | 440 | I | P | I | V | P | G | S | P | Q | Y | T | I | I | 88000 |
| 223 | 235 | A | R | L | G | L | Y | S | R | T | T | Q | Q | V | 88500 |
| 318 | 330 | K | S | I | G | A | K | V | H | Y | Y | Y | D | L | 94000 |
| 443 | 455 | A | G | D | F | Y | L | H | P | S | Y | Y | M | L | 97500 |
| 328 | 340 | Y | D | L | S | T | I | D | P | A | E | E | I | E | 102000 |
| 392 | 404 | G | Y | I | P | A | N | T | T | I | P | F | G | G | 102000 |
| 320 | 332 | I | G | A | K | V | H | Y | Y | Y | D | L | S | T | 110000 |
| 441 | 453 | A | D | A | G | D | F | Y | L | H | P | S | Y | Y | 110000 |
| 373 | 385 | F | I | T | D | T | S | T | T | P | V | P | S | V | 120000 |
| 109 | 121 | V | E | E | T | S | F | I | D | A | G | A | P | T | 125000 |
| 257 | 269 | Y | E | G | I | D | V | D | N | T | L | Y | F | S | 130000 |
| 354 | 366 | H | A | A | S | P | T | S | I | N | N | G | L | Y | 130000 |
| 60 | 72 | I | G | T | G | S | G | T | G | G | R | T | G | Y | 140000 |
| 105 | 117 | I | V | S | L | V | E | E | T | S | F | I | D | A | 150000 |
| 360 | 372 | S | I | N | N | G | L | Y | D | I | Y | A | D | D | 150000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|--------|
| 436 | 448 | Q | Y | T | I | I | A | D | A | G | D | F | Y | L | 150000 |
| 64 | 76 | S | G | T | G | G | R | T | G | Y | I | P | L | G | 160000 |
| 244 | 256 | T | T | P | T | K | L | I | T | Y | D | N | P | A | 160000 |
| 271 | 283 | N | D | N | S | I | N | I | A | P | D | P | D | F | 162000 |
| 167 | 179 | S | V | L | Q | P | P | T | P | A | E | T | G | G | 165000 |
| 54 | 66 | F | F | G | G | L | G | I | G | T | G | S | G | T | 175000 |
| 19 | 31 | Y | K | T | C | K | Q | A | G | T | C | P | P | D | 180000 |
| 176 | 188 | E | T | G | G | H | F | T | L | S | S | S | T | I | 180000 |
| 72 | 84 | Y | I | P | L | G | T | R | P | P | T | A | T | D | 190000 |
| 241 | 253 | A | F | V | T | T | P | T | K | L | I | T | Y | D | 190000 |
| 96 | 108 | D | P | V | G | P | S | D | P | S | I | V | S | L | 195000 |
| 393 | 405 | Y | I | P | A | N | T | T | I | P | F | G | G | A | 200000 |
| 230 | 242 | R | T | T | Q | Q | V | K | V | V | D | P | A | F | 205000 |
| 111 | 123 | E | T | S | F | I | D | A | G | A | P | T | S | V | 210000 |
| 124 | 136 | P | S | I | P | P | D | V | S | G | F | S | I | T | 210000 |
| 104 | 116 | S | I | V | S | L | V | E | E | T | S | F | I | D | 220000 |
| 357 | 369 | S | P | T | S | I | N | N | G | L | Y | D | I | Y | 220000 |
| 37 | 49 | E | G | K | T | I | A | E | Q | I | L | Q | Y | G | 222000 |
| 408 | 420 | I | P | L | V | S | G | P | D | I | P | I | N | I | 235000 |
| 130 | 142 | V | S | G | F | S | I | T | T | S | T | D | T | T | 240000 |
| 303 | 315 | Y | S | R | I | G | N | K | Q | T | L | R | T | R | 242000 |
| 398 | 410 | T | T | I | P | F | G | G | A | Y | N | I | P | L | 245000 |
| 401 | 413 | P | F | G | G | A | Y | N | I | P | L | V | S | G | 245000 |
| 193 | 205 | Y | E | E | I | P | M | D | T | F | I | V | S | T | 260000 |
| 245 | 257 | T | P | T | K | L | I | T | Y | D | N | P | A | Y | 265000 |
| 63 | 75 | G | S | G | T | G | G | R | T | G | Y | I | P | L | 270000 |
| 197 | 209 | P | M | D | T | F | I | V | S | T | N | P | N | T | 270000 |
| 194 | 206 | E | E | I | P | M | D | T | F | I | V | S | T | N | 280000 |
| 344 | 356 | I | T | P | S | T | Y | T | T | T | S | H | A | A | 292000 |
| 61 | 73 | G | T | G | S | G | T | G | G | R | T | G | Y | I | 295000 |
| 321 | 333 | G | A | K | V | H | Y | Y | Y | D | L | S | T | I | 312000 |
| 379 | 391 | T | T | P | V | P | S | V | P | S | T | S | L | S | 320000 |
| 304 | 316 | S | R | I | G | N | K | Q | T | L | R | T | R | S | 325000 |
| 385 | 397 | V | P | S | T | S | L | S | G | Y | I | P | A | N | 330000 |
| 59 | 71 | G | I | G | T | G | S | G | T | G | G | R | T | G | 340000 |
| 345 | 357 | T | P | S | T | Y | T | T | T | S | H | A | A | S | 350000 |
| 67 | 79 | G | G | R | T | G | Y | I | P | L | G | T | R | P | 360000 |
| 382 | 394 | V | P | S | V | P | S | T | S | L | S | G | Y | I | 365000 |
| 188 | 200 | I | S | T | H | N | Y | E | E | I | P | M | D | T | 372000 |
| 79 | 91 | P | P | T | A | T | D | T | L | A | P | V | R | P | 375000 |
| 11 | 23 | K | R | A | S | A | T | Q | L | Y | K | T | C | K | 382000 |
| 120 | 132 | P | T | S | V | P | S | I | P | P | D | V | S | G | 385000 |
| 107 | 119 | S | L | V | E | E | T | S | F | I | D | A | G | A | 400000 |
| 366 | 378 | Y | D | I | Y | A | D | D | F | I | T | D | T | S | 415000 |
| 189 | 201 | S | T | H | N | Y | E | E | I | P | M | D | T | F | 420000 |
| 145 | 157 | I | L | D | I | N | N | T | V | T | T | V | T | T | 440000 |
| 20 | 32 | K | T | C | K | Q | A | G | T | C | P | P | D | I | 442000 |
| 183 | 195 | L | S | S | S | T | I | S | T | H | N | Y | E | E | 450000 |
| 451 | 463 | S | Y | Y | M | L | R | K | R | R | K | R | L | P | 462000 |
| 10 | 22 | T | K | R | A | S | A | T | Q | L | Y | K | T | C | 470000 |
| 34 | 46 | P | K | V | E | G | K | T | I | A | E | Q | I | L | 490000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 136 | 148 | T | T | S | T | D | T | T | P | A | I | L | D | I | 490000 |
| 139 | 151 | T | D | T | T | P | A | I | L | D | I | N | N | T | 510000 |
| 118 | 130 | G | A | P | T | S | V | P | S | I | P | P | D | V | 545000 |
| 160 | 172 | N | P | T | F | T | D | P | S | V | L | Q | P | P | 545000 |
| 159 | 171 | N | N | P | T | F | T | D | P | S | V | L | Q | P | 575000 |
| 234 | 246 | Q | V | K | V | V | D | P | A | F | V | T | T | P | 585000 |
| 81 | 93 | T | A | T | D | T | L | A | P | V | R | P | P | L | 590000 |
| 177 | 189 | T | G | G | H | F | T | L | S | S | S | T | I | S | 590000 |
| 172 | 184 | P | T | P | A | E | T | G | G | H | F | T | L | S | 630000 |
| 426 | 438 | S | L | I | P | I | V | P | G | S | P | Q | Y | T | 630000 |
| 47 | 59 | Q | Y | G | S | M | G | V | F | F | G | G | L | G | 635000 |
| 362 | 374 | N | N | G | L | Y | D | I | Y | A | D | D | F | I | 640000 |
| 121 | 133 | T | S | V | P | S | I | P | P | D | V | S | G | F | 650000 |
| 341 | 353 | L | Q | T | I | T | P | S | T | Y | T | T | T | S | 665000 |
| 141 | 153 | T | T | P | A | I | L | D | I | N | N | T | V | T | 685000 |
| 310 | 322 | Q | T | L | R | T | R | S | G | K | S | I | G | A | 710000 |
| 406 | 418 | Y | N | I | P | L | V | S | G | P | D | I | P | I | 730000 |
| 338 | 350 | E | I | E | L | Q | T | I | T | P | S | T | Y | T | 732000 |
| 280 | 292 | D | P | D | F | L | D | I | V | A | L | H | R | P | 745000 |
| 294 | 306 | L | T | S | R | R | T | G | I | R | Y | S | R | I | 752000 |
| 166 | 178 | P | S | V | L | Q | P | P | T | P | A | E | T | G | 770000 |
| 242 | 254 | F | V | T | T | P | T | K | L | I | T | Y | D | N | 780000 |
| 384 | 396 | S | V | P | S | T | S | L | S | G | Y | I | P | A | 795000 |
| 356 | 368 | A | S | P | T | S | I | N | N | G | L | Y | D | I | 802000 |
| 155 | 167 | V | T | T | H | N | N | P | T | F | T | D | P | S | 810000 |
| 222 | 234 | V | A | R | L | G | L | Y | S | R | T | T | Q | Q | 860000 |
| 75 | 87 | L | G | T | R | P | P | T | A | T | D | T | L | A | 880000 |
| 440 | 452 | I | A | D | A | G | D | F | Y | L | H | P | S | Y | 910000 |
| 375 | 387 | T | D | T | S | T | T | P | V | P | S | V | P | S | 922000 |
| 9 | 21 | R | T | K | R | A | S | A | T | Q | L | Y | K | T | 945000 |
| 417 | 429 | P | I | N | I | T | D | Q | A | P | S | L | I | P | 952000 |
| 190 | 202 | T | H | N | Y | E | E | I | P | M | D | T | F | I | 982000 |
| 1 | 13 | M | R | H | K | R | S | A | K | R | T | K | R | A | 1000000 |
| 2 | 14 | R | H | K | R | S | A | K | R | T | K | R | A | S | 1000000 |
| 3 | 15 | H | K | R | S | A | K | R | T | K | R | A | S | A | 1000000 |
| 4 | 16 | K | R | S | A | K | R | T | K | R | A | S | A | T | 1000000 |
| 5 | 17 | R | S | A | K | R | T | K | R | A | S | A | T | Q | 1000000 |
| 6 | 18 | S | A | K | R | T | K | R | A | S | A | T | Q | L | 1000000 |
| 7 | 19 | A | K | R | T | K | R | A | S | A | T | Q | L | Y | 1000000 |
| 12 | 24 | R | A | S | A | T | Q | L | Y | K | T | C | K | Q | 1000000 |
| 13 | 25 | A | S | A | T | Q | L | Y | K | T | C | K | Q | A | 1000000 |
| 15 | 27 | A | T | Q | L | Y | K | T | C | K | Q | A | G | T | 1000000 |
| 18 | 30 | L | Y | K | T | C | K | Q | A | G | T | C | P | P | 1000000 |
| 21 | 33 | T | C | K | Q | A | G | T | C | P | P | D | I | I | 1000000 |
| 22 | 34 | C | K | Q | A | G | T | C | P | P | D | I | I | P | 1000000 |
| 23 | 35 | K | Q | A | G | T | C | P | P | D | I | I | P | K | 1000000 |
| 24 | 36 | Q | A | G | T | C | P | P | D | I | I | P | K | V | 1000000 |
| 25 | 37 | A | G | T | C | P | P | D | I | I | P | K | V | E | 1000000 |
| 26 | 38 | G | T | C | P | P | D | I | I | P | K | V | E | G | 1000000 |
| 27 | 39 | T | C | P | P | D | I | I | P | K | V | E | G | K | 1000000 |
| 28 | 40 | C | P | P | D | I | I | P | K | V | E | G | K | T | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 29 | 41 | P | P | D | I | I | P | K | V | E | G | K | T | I | 1000000 |
| 30 | 42 | P | D | I | I | P | K | V | E | G | K | T | I | A | 1000000 |
| 31 | 43 | D | I | I | P | K | V | E | G | K | T | I | A | E | 1000000 |
| 32 | 44 | I | I | P | K | V | E | G | K | T | I | A | E | Q | 1000000 |
| 33 | 45 | I | P | K | V | E | G | K | T | I | A | E | Q | I | 1000000 |
| 35 | 47 | K | V | E | G | K | T | I | A | E | Q | I | L | Q | 1000000 |
| 36 | 48 | V | E | G | K | T | I | A | E | Q | I | L | Q | Y | 1000000 |
| 40 | 52 | T | I | A | E | Q | I | L | Q | Y | G | S | M | G | 1000000 |
| 41 | 53 | I | A | E | Q | I | L | Q | Y | G | S | M | G | V | 1000000 |
| 45 | 57 | I | L | Q | Y | G | S | M | G | V | F | F | G | G | 1000000 |
| 48 | 60 | Y | G | S | M | G | V | F | F | G | G | L | G | I | 1000000 |
| 62 | 74 | T | G | S | G | T | G | G | R | T | G | Y | I | P | 1000000 |
| 65 | 77 | G | T | G | G | R | T | G | Y | I | P | L | G | T | 1000000 |
| 68 | 80 | G | R | T | G | Y | I | P | L | G | T | R | P | P | 1000000 |
| 69 | 81 | R | T | G | Y | I | P | L | G | T | R | P | P | T | 1000000 |
| 71 | 83 | G | Y | I | P | L | G | T | R | P | P | T | A | T | 1000000 |
| 73 | 85 | I | P | L | G | T | R | P | P | T | A | T | D | T | 1000000 |
| 74 | 86 | P | L | G | T | R | P | P | T | A | T | D | T | L | 1000000 |
| 76 | 88 | G | T | R | P | P | T | A | T | D | T | L | A | P | 1000000 |
| 77 | 89 | T | R | P | P | T | A | T | D | T | L | A | P | V | 1000000 |
| 78 | 90 | R | P | P | T | A | T | D | T | L | A | P | V | R | 1000000 |
| 80 | 92 | P | T | A | T | D | T | L | A | P | V | R | P | P | 1000000 |
| 82 | 94 | A | T | D | T | L | A | P | V | R | P | P | L | T | 1000000 |
| 83 | 95 | T | D | T | L | A | P | V | R | P | P | L | T | V | 1000000 |
| 85 | 97 | T | L | A | P | V | R | P | P | L | T | V | D | P | 1000000 |
| 86 | 98 | L | A | P | V | R | P | P | L | T | V | D | P | V | 1000000 |
| 87 | 99 | A | P | V | R | P | P | L | T | V | D | P | V | G | 1000000 |
| 88 | 100 | P | V | R | P | P | L | T | V | D | P | V | G | P | 1000000 |
| 89 | 101 | V | R | P | P | L | T | V | D | P | V | G | P | S | 1000000 |
| 90 | 102 | R | P | P | L | T | V | D | P | V | G | P | S | D | 1000000 |
| 92 | 104 | P | L | T | V | D | P | V | G | P | S | D | P | S | 1000000 |
| 94 | 106 | T | V | D | P | V | G | P | S | D | P | S | I | V | 1000000 |
| 95 | 107 | V | D | P | V | G | P | S | D | P | S | I | V | S | 1000000 |
| 97 | 109 | P | V | G | P | S | D | P | S | I | V | S | L | V | 1000000 |
| 98 | 110 | V | G | P | S | D | P | S | I | V | S | L | V | E | 1000000 |
| 99 | 111 | G | P | S | D | P | S | I | V | S | L | V | E | E | 1000000 |
| 100 | 112 | P | S | D | P | S | I | V | S | L | V | E | E | T | 1000000 |
| 101 | 113 | S | D | P | S | I | V | S | L | V | E | E | T | S | 1000000 |
| 102 | 114 | D | P | S | I | V | S | L | V | E | E | T | S | F | 1000000 |
| 108 | 120 | L | V | E | E | T | S | F | I | D | A | G | A | P | 1000000 |
| 114 | 126 | F | I | D | A | G | A | P | T | S | V | P | S | I | 1000000 |
| 115 | 127 | I | D | A | G | A | P | T | S | V | P | S | I | P | 1000000 |
| 116 | 128 | D | A | G | A | P | T | S | V | P | S | I | P | P | 1000000 |
| 117 | 129 | A | G | A | P | T | S | V | P | S | I | P | P | D | 1000000 |
| 119 | 131 | A | P | T | S | V | P | S | I | P | P | D | V | S | 1000000 |
| 122 | 134 | S | V | P | S | I | P | P | D | V | S | G | F | S | 1000000 |
| 123 | 135 | V | P | S | I | P | P | D | V | S | G | F | S | I | 1000000 |
| 125 | 137 | S | I | P | P | D | V | S | G | F | S | I | T | T | 1000000 |
| 126 | 138 | I | P | P | D | V | S | G | F | S | I | T | T | S | 1000000 |
| 127 | 139 | P | P | D | V | S | G | F | S | I | T | T | S | T | 1000000 |
| 129 | 141 | D | V | S | G | F | S | I | T | T | S | T | D | T | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 132 | 144 | G | F | S | I | T | T | S | T | D | T | T | P | A | 1000000 |
| 137 | 149 | T | S | T | D | T | T | P | A | I | L | D | I | N | 1000000 |
| 138 | 150 | S | T | D | T | T | P | A | I | L | D | I | N | N | 1000000 |
| 140 | 152 | D | T | T | P | A | I | L | D | I | N | N | T | V | 1000000 |
| 142 | 154 | T | P | A | I | L | D | I | N | N | T | V | T | T | 1000000 |
| 147 | 159 | D | I | N | N | T | V | T | T | V | T | T | H | N | 1000000 |
| 148 | 160 | I | N | N | T | V | T | T | V | T | T | H | N | N | 1000000 |
| 156 | 168 | T | T | H | N | N | P | T | F | T | D | P | S | V | 1000000 |
| 157 | 169 | T | H | N | N | P | T | F | T | D | P | S | V | L | 1000000 |
| 158 | 170 | H | N | N | P | T | F | T | D | P | S | V | L | Q | 1000000 |
| 162 | 174 | T | F | T | D | P | S | V | L | Q | P | P | T | P | 1000000 |
| 163 | 175 | F | T | D | P | S | V | L | Q | P | P | T | P | A | 1000000 |
| 164 | 176 | T | D | P | S | V | L | Q | P | P | T | P | A | E | 1000000 |
| 165 | 177 | D | P | S | V | L | Q | P | P | T | P | A | E | T | 1000000 |
| 168 | 180 | V | L | Q | P | P | T | P | A | E | T | G | G | H | 1000000 |
| 169 | 181 | L | Q | P | P | T | P | A | E | T | G | G | H | F | 1000000 |
| 170 | 182 | Q | P | P | T | P | A | E | T | G | G | H | F | T | 1000000 |
| 171 | 183 | P | P | T | P | A | E | T | G | G | H | F | T | L | 1000000 |
| 173 | 185 | T | P | A | E | T | G | G | H | F | T | L | S | S | 1000000 |
| 174 | 186 | P | A | E | T | G | G | H | F | T | L | S | S | S | 1000000 |
| 178 | 190 | G | G | H | F | T | L | S | S | S | T | I | S | T | 1000000 |
| 184 | 196 | S | S | S | T | I | S | T | H | N | Y | E | E | I | 1000000 |
| 187 | 199 | T | I | S | T | H | N | Y | E | E | I | P | M | D | 1000000 |
| 191 | 203 | H | N | Y | E | E | I | P | M | D | T | F | I | V | 1000000 |
| 192 | 204 | N | Y | E | E | I | P | M | D | T | F | I | V | S | 1000000 |
| 195 | 207 | E | I | P | M | D | T | F | I | V | S | T | N | P | 1000000 |
| 202 | 214 | I | V | S | T | N | P | N | T | V | T | S | S | T | 1000000 |
| 204 | 216 | S | T | N | P | N | T | V | T | S | S | T | P | I | 1000000 |
| 205 | 217 | T | N | P | N | T | V | T | S | S | T | P | I | P | 1000000 |
| 206 | 218 | N | P | N | T | V | T | S | S | T | P | I | P | G | 1000000 |
| 210 | 222 | V | T | S | S | T | P | I | P | G | S | R | P | V | 1000000 |
| 211 | 223 | T | S | S | T | P | I | P | G | S | R | P | V | A | 1000000 |
| 212 | 224 | S | S | T | P | I | P | G | S | R | P | V | A | R | 1000000 |
| 213 | 225 | S | T | P | I | P | G | S | R | P | V | A | R | L | 1000000 |
| 214 | 226 | T | P | I | P | G | S | R | P | V | A | R | L | G | 1000000 |
| 215 | 227 | P | I | P | G | S | R | P | V | A | R | L | G | L | 1000000 |
| 216 | 228 | I | P | G | S | R | P | V | A | R | L | G | L | Y | 1000000 |
| 217 | 229 | P | G | S | R | P | V | A | R | L | G | L | Y | S | 1000000 |
| 218 | 230 | G | S | R | P | V | A | R | L | G | L | Y | S | R | 1000000 |
| 219 | 231 | S | R | P | V | A | R | L | G | L | Y | S | R | T | 1000000 |
| 221 | 233 | P | V | A | R | L | G | L | Y | S | R | T | T | Q | 1000000 |
| 227 | 239 | L | Y | S | R | T | T | Q | Q | V | K | V | V | D | 1000000 |
| 228 | 240 | Y | S | R | T | T | Q | Q | V | K | V | V | D | P | 1000000 |
| 229 | 241 | S | R | T | T | Q | Q | V | K | V | V | D | P | A | 1000000 |
| 231 | 243 | T | T | Q | Q | V | K | V | V | D | P | A | F | V | 1000000 |
| 232 | 244 | T | Q | Q | V | K | V | V | D | P | A | F | V | T | 1000000 |
| 235 | 247 | V | K | V | V | D | P | A | F | V | T | T | P | T | 1000000 |
| 237 | 249 | V | V | D | P | A | F | V | T | T | P | T | K | L | 1000000 |
| 238 | 250 | V | D | P | A | F | V | T | T | P | T | K | L | I | 1000000 |
| 239 | 251 | D | P | A | F | V | T | T | P | T | K | L | I | T | 1000000 |
| 243 | 255 | V | T | T | P | T | K | L | I | T | Y | D | N | P | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 250 | 262 | I | T | Y | D | N | P | A | Y | E | G | I | D | V | 1000000 |
| 251 | 263 | T | Y | D | N | P | A | Y | E | G | I | D | V | D | 1000000 |
| 252 | 264 | Y | D | N | P | A | Y | E | G | I | D | V | D | N | 1000000 |
| 253 | 265 | D | N | P | A | Y | E | G | I | D | V | D | N | T | 1000000 |
| 254 | 266 | N | P | A | Y | E | G | I | D | V | D | N | T | L | 1000000 |
| 256 | 268 | A | Y | E | G | I | D | V | D | N | T | L | Y | F | 1000000 |
| 260 | 272 | I | D | V | D | N | T | L | Y | F | S | S | N | D | 1000000 |
| 261 | 273 | D | V | D | N | T | L | Y | F | S | S | N | D | N | 1000000 |
| 262 | 274 | V | D | N | T | L | Y | F | S | S | N | D | N | S | 1000000 |
| 267 | 279 | Y | F | S | S | N | D | N | S | I | N | I | A | P | 1000000 |
| 269 | 281 | S | S | N | D | N | S | I | N | I | A | P | D | P | 1000000 |
| 270 | 282 | S | N | D | N | S | I | N | I | A | P | D | P | D | 1000000 |
| 272 | 284 | D | N | S | I | N | I | A | P | D | P | D | F | L | 1000000 |
| 274 | 286 | S | I | N | I | A | P | D | P | D | F | L | D | I | 1000000 |
| 275 | 287 | I | N | I | A | P | D | P | D | F | L | D | I | V | 1000000 |
| 276 | 288 | N | I | A | P | D | P | D | F | L | D | I | V | A | 1000000 |
| 277 | 289 | I | A | P | D | P | D | F | L | D | I | V | A | L | 1000000 |
| 278 | 290 | A | P | D | P | D | F | L | D | I | V | A | L | H | 1000000 |
| 279 | 291 | P | D | P | D | F | L | D | I | V | A | L | H | R | 1000000 |
| 283 | 295 | F | L | D | I | V | A | L | H | R | P | A | L | T | 1000000 |
| 286 | 298 | I | V | A | L | H | R | P | A | L | T | S | R | R | 1000000 |
| 287 | 299 | V | A | L | H | R | P | A | L | T | S | R | R | T | 1000000 |
| 288 | 300 | A | L | H | R | P | A | L | T | S | R | R | T | G | 1000000 |
| 289 | 301 | L | H | R | P | A | L | T | S | R | R | T | G | I | 1000000 |
| 290 | 302 | H | R | P | A | L | T | S | R | R | T | G | I | R | 1000000 |
| 291 | 303 | R | P | A | L | T | S | R | R | T | G | I | R | Y | 1000000 |
| 293 | 305 | A | L | T | S | R | R | T | G | I | R | Y | S | R | 1000000 |
| 295 | 307 | T | S | R | R | T | G | I | R | Y | S | R | I | G | 1000000 |
| 296 | 308 | S | R | R | T | G | I | R | Y | S | R | I | G | N | 1000000 |
| 297 | 309 | R | R | T | G | I | R | Y | S | R | I | G | N | K | 1000000 |
| 298 | 310 | R | T | G | I | R | Y | S | R | I | G | N | K | Q | 1000000 |
| 300 | 312 | G | I | R | Y | S | R | I | G | N | K | Q | T | L | 1000000 |
| 302 | 314 | R | Y | S | R | I | G | N | K | Q | T | L | R | T | 1000000 |
| 305 | 317 | R | I | G | N | K | Q | T | L | R | T | R | S | G | 1000000 |
| 306 | 318 | I | G | N | K | Q | T | L | R | T | R | S | G | K | 1000000 |
| 307 | 319 | G | N | K | Q | T | L | R | T | R | S | G | K | S | 1000000 |
| 308 | 320 | N | K | Q | T | L | R | T | R | S | G | K | S | I | 1000000 |
| 311 | 323 | T | L | R | T | R | S | G | K | S | I | G | A | K | 1000000 |
| 313 | 325 | R | T | R | S | G | K | S | I | G | A | K | V | H | 1000000 |
| 314 | 326 | T | R | S | G | K | S | I | G | A | K | V | H | Y | 1000000 |
| 316 | 328 | S | G | K | S | I | G | A | K | V | H | Y | Y | Y | 1000000 |
| 319 | 331 | S | I | G | A | K | V | H | Y | Y | Y | D | L | S | 1000000 |
| 323 | 335 | K | V | H | Y | Y | Y | D | L | S | T | I | D | P | 1000000 |
| 327 | 339 | Y | Y | D | L | S | T | I | D | P | A | E | E | I | 1000000 |
| 329 | 341 | D | L | S | T | I | D | P | A | E | E | I | E | L | 1000000 |
| 330 | 342 | L | S | T | I | D | P | A | E | E | I | E | L | Q | 1000000 |
| 332 | 344 | T | I | D | P | A | E | E | I | E | L | Q | T | I | 1000000 |
| 333 | 345 | I | D | P | A | E | E | I | E | L | Q | T | I | T | 1000000 |
| 334 | 346 | D | P | A | E | E | I | E | L | Q | T | I | T | P | 1000000 |
| 335 | 347 | P | A | E | E | I | E | L | Q | T | I | T | P | S | 1000000 |
| 336 | 348 | A | E | E | I | E | L | Q | T | I | T | P | S | T | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 340 | 352 | E | L | Q | T | I | T | P | S | T | Y | T | T | T | 1000000 |
| 343 | 355 | T | I | T | P | S | T | Y | T | T | T | S | H | A | 1000000 |
| 351 | 363 | T | T | S | H | A | A | S | P | T | S | I | N | N | 1000000 |
| 352 | 364 | T | S | H | A | A | S | P | T | S | I | N | N | G | 1000000 |
| 353 | 365 | S | H | A | A | S | P | T | S | I | N | N | G | L | 1000000 |
| 355 | 367 | A | A | S | P | T | S | I | N | N | G | L | Y | D | 1000000 |
| 361 | 373 | I | N | N | G | L | Y | D | I | Y | A | D | D | F | 1000000 |
| 365 | 377 | L | Y | D | I | Y | A | D | D | F | I | T | D | T | 1000000 |
| 368 | 380 | I | Y | A | D | D | F | I | T | D | T | S | T | T | 1000000 |
| 369 | 381 | Y | A | D | D | F | I | T | D | T | S | T | T | P | 1000000 |
| 370 | 382 | A | D | D | F | I | T | D | T | S | T | T | P | V | 1000000 |
| 376 | 388 | D | T | S | T | T | P | V | P | S | V | P | S | T | 1000000 |
| 378 | 390 | S | T | T | P | V | P | S | V | P | S | T | S | L | 1000000 |
| 380 | 392 | T | P | V | P | S | V | P | S | T | S | L | S | G | 1000000 |
| 381 | 393 | P | V | P | S | V | P | S | T | S | L | S | G | Y | 1000000 |
| 383 | 395 | P | S | V | P | S | T | S | L | S | G | Y | I | P | 1000000 |
| 389 | 401 | S | L | S | G | Y | I | P | A | N | T | T | I | P | 1000000 |
| 390 | 402 | L | S | G | Y | I | P | A | N | T | T | I | P | F | 1000000 |
| 394 | 406 | I | P | A | N | T | T | I | P | F | G | G | A | Y | 1000000 |
| 395 | 407 | P | A | N | T | T | I | P | F | G | G | A | Y | N | 1000000 |
| 396 | 408 | A | N | T | T | I | P | F | G | G | A | Y | N | I | 1000000 |
| 399 | 411 | T | I | P | F | G | G | A | Y | N | I | P | L | V | 1000000 |
| 403 | 415 | G | G | A | Y | N | I | P | L | V | S | G | P | D | 1000000 |
| 405 | 417 | A | Y | N | I | P | L | V | S | G | P | D | I | P | 1000000 |
| 407 | 419 | N | I | P | L | V | S | G | P | D | I | P | I | N | 1000000 |
| 410 | 422 | L | V | S | G | P | D | I | P | I | N | I | T | D | 1000000 |
| 411 | 423 | V | S | G | P | D | I | P | I | N | I | T | D | Q | 1000000 |
| 412 | 424 | S | G | P | D | I | P | I | N | I | T | D | Q | A | 1000000 |
| 413 | 425 | G | P | D | I | P | I | N | I | T | D | Q | A | P | 1000000 |
| 415 | 427 | D | I | P | I | N | I | T | D | Q | A | P | S | L | 1000000 |
| 419 | 431 | N | I | T | D | Q | A | P | S | L | I | P | I | V | 1000000 |
| 420 | 432 | I | T | D | Q | A | P | S | L | I | P | I | V | P | 1000000 |
| 421 | 433 | T | D | Q | A | P | S | L | I | P | I | V | P | G | 1000000 |
| 422 | 434 | D | Q | A | P | S | L | I | P | I | V | P | G | S | 1000000 |
| 424 | 436 | A | P | S | L | I | P | I | V | P | G | S | P | Q | 1000000 |
| 427 | 439 | L | I | P | I | V | P | G | S | P | Q | Y | T | I | 1000000 |
| 429 | 441 | P | I | V | P | G | S | P | Q | Y | T | I | I | A | 1000000 |
| 430 | 442 | I | V | P | G | S | P | Q | Y | T | I | I | A | D | 1000000 |
| 432 | 444 | P | G | S | P | Q | Y | T | I | I | A | D | A | G | 1000000 |
| 434 | 446 | S | P | Q | Y | T | I | I | A | D | A | G | D | F | 1000000 |
| 439 | 451 | I | I | A | D | A | G | D | F | Y | L | H | P | S | 1000000 |
| 442 | 454 | D | A | G | D | F | Y | L | H | P | S | Y | Y | M | 1000000 |
| 447 | 459 | Y | L | H | P | S | Y | Y | M | L | R | K | R | R | 1000000 |
| 448 | 460 | L | H | P | S | Y | Y | M | L | R | K | R | R | K | 1000000 |
| 449 | 461 | H | P | S | Y | Y | M | L | R | K | R | R | K | R | 1000000 |
| 452 | 464 | Y | Y | M | L | R | K | R | R | K | R | L | P | Y | 1000000 |
| 453 | 465 | Y | M | L | R | K | R | R | K | R | L | P | Y | F | 1000000 |
| 454 | 466 | M | L | R | K | R | R | K | R | L | P | Y | F | F | 1000000 |
| 455 | 467 | L | R | K | R | R | K | R | L | P | Y | F | F | S | 1000000 |
| 456 | 468 | R | K | R | R | K | R | L | P | Y | F | F | S | D | 1000000 |
| 457 | 469 | K | R | R | K | R | L | P | Y | F | F | S | D | V | 1000000 |

| | | | | | | | | | | | | | | | |
|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| 458 | 470 | R | R | K | R | L | P | Y | F | F | S | D | V | S | 1000000 |
| 459 | 471 | R | K | R | L | P | Y | F | F | S | D | V | S | L | 1000000 |
| 461 | 473 | R | L | P | Y | F | F | S | D | V | S | L | A | A | 1000000 |